MDAH-2021

Virtual from Guelph, ON, CA

June 1st-3rd 2021

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WELCOMING REMARKS

Thank you for your patience during these unprecedented times as we move our conference from our city of Guelph, ON to your computers. We welcome you to our shared virtual space. We are pleased to have the privilege to host the 13th International Symposium on Marek's Disease and Avian Herpesvirus, and for the first time, online.

Our international symposium has esteemed experts and students from across the globe. We have planned our interactive events with the hopes to join our researchers across time zones into one harmonized symposium.

We request everyone attend the first NA/EU reception/social hour where we will be explaining the schedule and format of our conference, how to interact with the Chat function and live sessions, and to get orientated on our virtual platform on the main stage.

Thank you again for your support during these unprecedented times, and we hope you enjoy what we have to offer!

Important

Attendees are expected to **watch the abstract presentations before the corresponding live discussion event**. We will *not* be streaming the presentations during the live discussion events. The live discussion events are only for Q&A. You can watch the abstract presentations in Expo booths. Please use the Chat functions to ask questions. The session moderator will collect questions and voice your questions during the live discussion events.

GENERAL INFORMATION

HOPIN

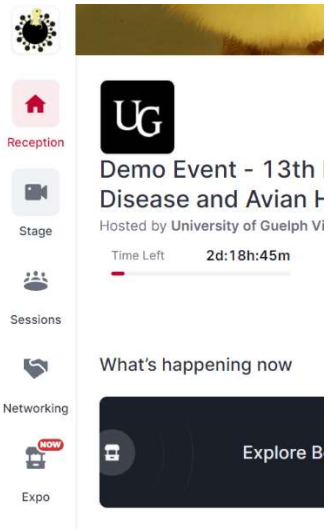
For the first time, the International Symposium on Marek's Disease and Avian Herpesvirus will be held online and virtually. Hopin is the online platform that will host our conference. Hopin is fully compatible on a desktop (web) on Google Chrome and Mozilla Firefox. If you are using a mobile device, the downloadable mobile app will provide you with the best experience.

MDAH video for full walkthrough of the platform: https://youtu.be/w3whDP5TVqg

Logging into Hopin

For information on magic links from Hopin: https://hopin.zendesk.com/hc/en-gb/articles/360059590591-How-to-use-Magic-Links-as-an-Attendee-Speaker-or-Moderator

Stages, Sessions, Networking, and Expo Booths



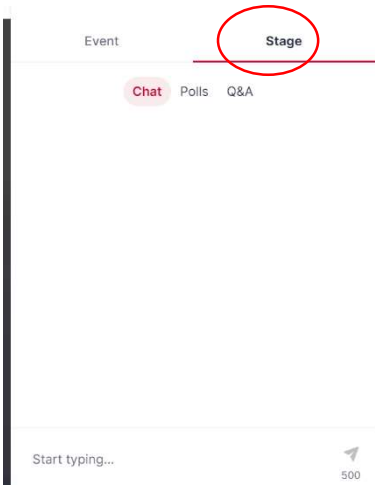
- Reception: This is where the schedule can be found.
- Main stage: where the keynote and industry speakers will be providing their live lectures.
- Sessions: where live discussion sessions will occur, and also where social/group-networking rooms where be hosted.
- The networking area: you will be paired up with another attendee for 1:1 networking.
- Expo booths: will host the Hopin support/troubleshooting booth, as well as booths for abstract presentation videos with a chat for any questions. Vendor booths will host representatives from our generous sponsors.

MDAH video for explanation of locations: https://youtu.be/jftsaueqzbkv

Where to Watch Keynote and Industry Presenters

The keynote and industry speakers will be presenting live from the stage. There should be a side bar on the left where you can click "Stage."

You can ask questions in the chat on the right-hand side. If you are asking a question to the speaker, please make sure to use the Chat function in the Stage and *not the Event*.



Socialization opportunities

In the sessions area, there will be networking rooms. In these networking rooms, you are able to video conference with up to 5 other people, so you can get to know others in small groups.

For one-on-one networking, the networking room will be open for short video conferencing calls of 3 minutes. A timer will be shown. The call will end once the timer is done. Once you click the "ready" button, the system will pair you up with another attendee who is also ready. After you have met, you will be able to exchange contact information using the "connect" button in the top right corner of the screen.

MDAH video explanation of socialization opportunities: https://youtu.be/tZ2svYFjyRs

For more information on networking areas, please visit: https://hopin.zendesk.com/hc/en-gb/articles/360056527831-Networking-FAQs

Time zones, Discussion boards, and Live Discussion Events

We are excited to welcome our guests from all around the world! Due to time differences, we will be offering two sets of sessions targeted towards guests in either North American and European time zones (NA/EU), or Asian and Australian time zones (AS/AU). In our schedules, we have designated NA/EU sessions as orange and AS/AU sessions as blue.

This year, MDAH2021 is hosted out of Guelph, Ontario, Canada which is in the Eastern Daylight Time zone on June 1st-3rd.

Similar to last year, we have 6 different topics that we have grouped the abstracts into (below). Most topics will also have 2 panels per topic.

- Epidemiology and diagnosis (2 panels)
- Immunology (2 panels)
- ILT vaccination (1 panel)
- Marek's disease vaccination (1 panel, will occur after ILT vaccination)
- Molecular virology (2 panels) and
- Pathogenesis and virus-cell interactions (2 panels).

Each panel will have its own booth where all corresponding abstract video presentations will be available to view. All attendees should watch the corresponding video presentations before the live discussion session. The videos will be available on YouTube or in Expo Booths on our platform (opening May 31st).

YouTube links for the playlists are available here:

Epidemiology and diagnosis panel 1:
https://www.youtube.com/watch?v=dqT0CwokdSg&list=PLE_Y_Amagkc3LVz0JaWNt4ZHG4yYmETWC
Epidemiology and diagnosis panel 2:
https://www.youtube.com/watch?v=RnVIPHrcHr4&list=PLE_Y_Amagkc02aXBZZ7qeEhJKv7RWPN0M

Immunology panel 1:
https://www.youtube.com/watch?v=rVClfR4eOm4&list=PLE_Y_Amagkc1yjBvgGF35eq_Hw-0tz0nP
Immunology panel 2:
https://www.youtube.com/watch?v=YklJEfTzRKY&list=PLE_Y_Amagkc2YbTnQ1eVATbOCcfzH4VaK

AS/AU panel 1 virus related factors:
https://www.youtube.com/watch?v=GulDJoHGT-I&list=PLE_Y_Amagkc3ZaJ5XO3zIqmOEtGLjZCIJ

ILT vaccination:
https://www.youtube.com/watch?v=8-aLYn_8blE&list=PLE_Y_Amagkc1wi3W6xyFUUZNp7rCQLnvC
MDV vaccination:
https://www.youtube.com/watch?v=nHatKTSlD54&list=PLE_Y_Amagkc3NHGPJXulpr-DC71rYpdoY

AS/AU panel 2 related field works:
https://www.youtube.com/watch?v=9uq_rgPcX0Q&list=PLE_Y_Amagkc3VjGkUHUbGdbT3ua8lmmgj

Molecular virology panel 1:
https://www.youtube.com/watch?v=djF-HmChNMQ&list=PLE_Y_Amagkc0ha9UNFfyQHAbBXwQ5wnEB
Molecular virology panel 2:
https://www.youtube.com/watch?v=bkTjDTmmAtI&list=PLE_Y_Amagkc15O-sMI6IhHZPNoqSdeBZ2

Pathogenesis and virus-cell interactions panel 1:
https://www.youtube.com/watch?v=JybTevn0STc&list=PLE_Y_Amagkc3PAf69jha8LNru0AtFuZJk
Pathogenesis and virus-cell interactions panel 2:
https://www.youtube.com/watch?v=QvxJCa5gTUM&list=PLE_Y_Amagkc2KYCVtrHv5zVlS5MWoflVp

Asking questions before the event/Chat function

Also in the booth will be a Chat for written questions. We highly encourage you to send any questions you have about presentations into the Chat.

It is extremely important to send questions into the Chat for discussions you may not be able to attend live. Moderators for each live panel will collect questions from the Chat and be the voice for those who cannot make the live event.

MDAH video on where to send questions for abstract presenters: https://youtu.be/33XY81fqzzE

Live Discussion Sessions

The live discussion sessions will occur in Sessions.

Each topic has its own session room. Panel #2 for each topic will occur in the same session room after Panel #1. All live events will be recorded and posted shortly after for those who cannot attend certain live events. Attendees of the live event are encouraged to ask live questions as well!

If after the live event you still have questions for the presenters, you are welcome to submit the new question into the Chat. If this is the case, please indicate that it is a *new* question. Presenters will be encouraged to check the discussion board after their live event and answer any new questions not answered in the live discussion session.

Bilibili

All video presentations will be uploaded to YouTube and be posted as "unlisted" so they can only be found by our links and cannot be found by search. Because YouTube is not available in China, we will be posting all videos to a similar platform available in China called Bilibili. Our guests from China will have access to the videos on Bilibili. All other guests will watch recordings on Hopin from embedded YouTube links.

We will delete all presentations off Bilibili after the event ends. Our Bilibili links (and YouTube links can be found here: https://www.mdah2020.com/how-to-use-hopin).

Awards

With the generous support of our sponsors, we are delighted to offer awards to the best presentations, as determined by a panel of judges.

The Graduate Student Presentation Awards committee judging will be anonymous. A Google forms with a standard scoring matrix will be used to evaluate each presentation. Judges will not receive forms for any of their students or lab members.

To be eligible for a graduate student award, the student must be present at the live discussion session.

The awards committee is composed of:
Dr. Benedikt Kaufer,
Dr. Venu Nair,
Dr. Maricarmen Garcia,
Dr. Caroline Denevsre,
Dr. Michel Bublot,
Dr. Shiro Murata,
Dr. Hans Cheng, and
Dr. Mark Parcells

Issues with Hopin?

If you run into any issues with Hopin, do not hesitate to ask for help in the Hopin Technical Support Booth in the Expo Booths.

For any general questions regarding the conference itself, please reach out to:
Alice Wang at mdah.symposium@uoguelph.ca

MDAH YouTube channel for exploring the Hopin platform:
https://www.youtube.com/channel/UCGtGEyQ-oanVOEXDLoefCeQ

SPONSORS

We are grateful to the following organizations for their generous support of our conference:

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Boehringer Ingelheim

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Program Summary

Notes: Participants are welcome to join any events. Events are only designated AS/AU and NA/EU to reflect convenience of attending regarding time zones.
Blue indicates events targeted towards guests joining from Asia and Australia (AS/AU).
Orange indicates events targeted towards guests joining from North America and Europe (NA/EU).

We highly encourage everyone to attend the first NA/EU Reception and Social. We will be explaining how to interact on the discussion boards and live discussions, and the conference format.

Conference participants are expected to watch the corresponding abstract presentations and submit any questions to the discussion boards before the live discussion event.

MDAH 2021 Program Summary					
Tuesday, June 1st - Guelph Time (EST - North America)			Start Time Equivalencies		
	Time	Event	Paris	Beijing	Melbourne
	07:30 – 08:30	**NA/EU Reception and Social** Location: Reception, Stage, Sessions (Networking Room)	13:30 06/01	19:30 06/01	21:30 06/01
	08:30 – 09:30	**Keynote Speaker: Dr. Wolfgang Hammerschmidt** Moderated by: Dr. Caroline Denesvre Sponsored by: Boehringer Ingelheim Ltd. Location: Stage	14:30 06/01	20:30 06/01	22:30 06/01
	09:30 – 10:15	**Industry speaker: Dr. Michel Bublot** Moderated by: Dr. Venugopal Nair Sponsored by: Aviagen Group Location: Stage	15:30 06/01	21:30 06/01	23:30 06/01
	10:15 – 11:45	**Concurrent NA/EU Live Boehringer Ingelheim Ltd. Discussions** Epidemiology and Diagnosis Location: Sessions Moderated by: Dr. Isabel Gimeno and Dr. John Dunn Immunology Location: Sessions Moderated by: Dr. Shayan Sharif and Dr. Masahiro Niikura	16:15 06/01	22:15 06/01	00:15 06/02
	11:45 – 12:45	**Networking and Reception** Location: Sessions (Networking Room), Networking, and Expo Booths for our Sponsors	17:45 06/01	23:45 06/01	01:15 06/02

				– June 2nd	– June 2nd
	19:30 – 20:30	**AS/AU Reception and Social** Location: Reception, Stage, Sessions (Networking Rooms)	01:30 06/02	07:30 06/02	09:30 06/02
	20:30 – 22:00	**Concurrent AS/AU Live Boehringer Ingelheim Ltd. Discussions** Related Virus-Factors: Sessions Moderated by: Dr. Stephen Walkden-Brown and Dr. Aijian Qin	02:30 06/02	08:30 06/02	10:30 06/02
	22:00 – 23:00	**Networking and Reception** Location: Sessions (Networking Room), Networking, and Expo Booths for our Sponsors	04:00 06/02	10:00 06/02	12:00 06/02
		*Watch Pre-Recorded Keynote Presentations: Dr. Wolfgang Hammerschmidt, Dr. Michel Bublot, and Dr. Helen Sang			

Wednesday, June 2nd – Guelph Time (EST – North America)			**Start Time Equivalencies**		
	Time	Event	Paris	Beijing – June 2nd	Melbourne – June 2nd
	07:30 – 08:30	**NA/EU Reception and Social** Location: Reception, Sessions (Networking Rooms)	13:30 06/02	19:30 06/02	21:30 06/02
	08:30 – 09:30	**Keynote Speaker: Dr. Blossom Damania** Moderated by: Dr. Karel (Ton) Schat Sponsored by: Merck/MSD & Co. Inc. Location: Stage	14:30 06/02	20:30 06/02	22:30 06/02
	09:30 – 10:15	**Industry Speaker: Dr. Guillermo Zavala** Moderated by: Dr. Maricarmen Garcia Location: Stage	15:30 06/02	21:30 06/02	23:30 06/02
	10:15 – 11:45	**Concurrent NA/EU Live Merck/MSD & Co. Inc. discussions** ILT Vaccination: Sessions Moderated by: Dr. Maricarmen Garcia Marek's Vaccination: Sessions Moderated by: Dr. Stephen Spatz	16:15 06/02	22:15 06/02	00:15 06/03
	11:45 – 12:45	**Networking and Reception** Location: Sessions (Networking Room), Networking, and Expo Booths for our Sponsors	17:45 06/02	23:45 06/02	01:45 06/03

				– June 3rd	– June 3rd
	19:30 – 20:30	**AS/AU Reception and Social** Location: Reception, Stage, and Sessions (Networking Room)	01:30 06/03	07:30 06/03	09:30 06/03
	20:30 – 22:00	**Concurrent AS/AU Live Merck/MSD & Co. Inc. discussions** Related Field Works: Sessions Moderated by: Dr. Joanne Devlin	02:30 06/03	08:30 06/03	10:30 06/03
	22:00 – 23:00	**Networking and Reception** Location: Sessions (Networking Rooms), Networking, and Expo Booths for our Sponsors	04:00 06/03	10:00 06/03	12:00 06/03
		*Watch Pre-Recorded Keynote Presentations: Dr. Blossom Damania and Dr. Guillermo Zavala			
Thursday, June 3rd – Guelph Time (EST – North America)			**Start Time Equivalencies**		
	Time	Event	Paris	Beijing – June 3rd	Melbourne – June 3rd
	07:30 – 09:00	**Concurrent NA/EU Ceva Animal Health Inc. Live discussions** Molecular Virology: Sessions Moderated by: Dr. Jiuzhou (John) Song and Dr. Sanjay Reddy Pathogenesis and Virus-cell Interactions: Sessions Moderated by: Dr. Ben Kaufer and Dr. Mark Parcells	13:30 06/03	19:30 06/03	21:30 06/03
	09:00 – 10:00	**Awards and Closing Remarks** Location: Stage	15:00 06/03	21:00 06/03	23:00 06/03
	10:00 – 11:00	**Networking and Reception** Location: Sessions (Networking Room), Networking, and Expo Booths for our Sponsors	16:00 06/03	22:00 06/03	00:00 06/04
		*Watch Pre-Recorded Keynote Presentation: Dr. Helen Sang. Sponsored by: Ceva Animal Health Inc.			

Live Keynote Speaker: Dr. Wolfgang Hammerschmidt. Sponsored by Boehringer Ingelheim

Helmholtz Centre Munich, German Research Center for Environment and Health

Moderated by: Dr. Caroline Denesvre

Dr. Wolfgang Hammerschmidt is a trained veterinarian. He studied at the Freie University Berlin and obtained his PhD in Microbiology. He then switched to bovine and equine herpesviruses as a postdoc in virology, moved to the US in 1987 and stayed with Bill Sugden in Madison, WI, University of Wisconsin, McArdle Laboratory for Cancer Research, for three years. Thanks to Bill, he discovered his interest in Epstein-Barr Virus and its extremely rich biology. Dr. Hammerschmidt then moved back to Germany and ended up in Bavaria and its capital Munich in 1990. Since this time, he has worked at the Helmholtz Center Munich – German Research Center for Environmental Health as head of an independent research unit.

His main focus is still Epstein-Barr virus and its target cells, human B lymphocytes. [But he also touched on Marek Disease Virus in 1993, the two references that evolved from this endeavor will be listed below!] Over the years he has developed a deep interest in EBV's genetics and its latent infection in human B cells. These activities involve cell biology, immunology and – surprise – virology of gamma-herpesviruses. Currently, part of his team is also very much engaged in clinical development of an EB virus-like particle vaccine to prevent Infectious Mononucleosis and its sequelae in adolescents and young adults.

Delecluse HJ, Schüller S, Hammerschmidt W (1993) Latent Marek's disease virus can be activated from its chromosomally integrated state in herpesvirus-transformed lymphoma cells. EMBO J 12:3277–3286

Delecluse HJ, Hammerschmidt W (1993) Status of Marek's disease virus in established lymphoma cell lines: herpesvirus integration is common. J Virol 67:82–92,

FIRST DAYS IN THE LIFE OF HUMAN B-LYMPHOCYTES INFECTED WITH EPSTEIN-BARR VIRUS

Paulin Mrozek-Gorska[1], Alexander Buschle[1], Dagmar Pich[1], Thomas Schwarzmayr[2], Ron Fechtner[3,4], Antonio Scialdone[3,4,5], and Wolfgang Hammerschmidt[1]
[1]Research Unit Gene Vectors, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health and German Center for Infection Research, Munich, Germany
[2]Institute of Human Genetics, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Neuherberg, Germany
[3]Institute of Epigenetics and Stem Cells, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Munich, Germany
[4]Institute of Computational Biology, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Neuherberg, Germany
[5]Institute of Functional Epigenetics, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Neuherberg, Germany

Epstein–Barr virus (EBV) is a human tumor virus and a model of herpesviral latency similar to MDV. EBV efficiently infects resting human B lymphocytes and induces their continuous proliferation *in vitro*, which mimics certain aspects of EBV's oncogenic potential *in vivo*. How lymphoblastoid cell lines (LCLs) evolve from the infected lymphocytes is uncertain. We conducted a systematic time-resolved longitudinal study of cellular functions and transcriptional profiles of newly infected naive B lymphocytes from human donors. EBV reprograms the cells comprehensively and globally. Rapid and extensive transcriptional changes occur within 24 h and precede any metabolic and phenotypic changes. Within 72 h, the virus activates the cells, changes their phenotypes with respect to cell size, RNA, and protein content, and induces metabolic pathways to cope with the increased demand for energy, supporting an efficient cell cycle entry on day 3 post infection. The transcriptional program that EBV initiates consists of 3 waves of clearly discernable clusters of cellular genes that peak on day 2, 3, or 4 and regulate RNA synthesis, metabolic pathways, and cell division, respectively. Upon onset of cell doublings on day 4, the cellular transcriptome appears to be completely reprogrammed to support the proliferating cells. More than 11,000 genes are regulated upon EBV infection as naive B cells exit quiescence to enter a germinal center-like differentiation program, which culminates in immortalized, proliferating cells that partially resemble plasmablasts and early plasma cells.

Live Industry Speaker: Dr. Michel Bublot. Sponsored by: The Aviagen Group

R&D Global Project Leader, Boehringer Ingelheim
Moderated by: Dr. Venugopal Nair

Michel Bublot is a veterinarian with a PhD in Virology from University of Liège (Belgium). He has been working in R&D for Rhône-Mérieux, Merial and now, Boehringer Ingelheim for more than 27 years. He has been heavily involved in the research and development of bivalent and trivalent HVT vector vaccines against IBD, ND and ILT, as well as in a new genetically modified serotype 1 Marek's disease vaccine. He is currently a Global Project Leader and is based in Lyon in France.

The presentation will cover practical aspects of MD vaccination with the different types of vaccines and major causes of vaccine failures

APPLIED ASPECTS OF MAREK'S DISEASE VACCINATION

M. Bublot[1]
[1]*Boehringer Ingelheim Animal Health, Global Innovation, Saint-Priest, France*

Classical Marek's disease (MD) vaccines based on serotypes 1 (*e.g.* Rispens or CVI988), 2 (*e.g.* SB-1) and 3 (herpesvirus of turkey (HVT)) have been developed 40 to 50 years ago and are still widely used today to control MD. In the last 15 years, new types of vaccines have reached the market : HVT vectored vaccines and genetically-modified serotype 1 vaccines. They may share different biological properties from those of classical vaccines. However, MD outbreak still occurs occasionally for multiple reasons. One of the key success factor of MD vaccination is the strict respect of the cold chain for transport and storage of vaccine. Heat shock may cause a severe drop in vaccine titer without signs of thawing. Vaccine thawing and reconstitution need to be done by trained hatchery personnel in a clean environment. Additives mixed with MD vaccine may impact the viral titer and should be checked properly under the supervision of veterinarians. *In ovo* or sub-cutaneous administration must be performed using clean, well calibrated and maintained equipment. Off label double dosage and/or revaccination are sometimes performed to increase vaccine coverage. The health status of the chick is also critical to induce a rapid onset of immunity. Vertical transmission of agents such as chicken infectious anemia virus may severely impact the protection induced by MD vaccine. Periodic auditing of hatchery MD vaccine handling, preparation and administration should be done to assure the constant respect of good practices. Field monitoring of vaccination may be performed but may give inconsistent results.

Live Boehringer Ingelheim Discussions Session 1

Session 1: Epidemiology and Diagnosis Panel 1

Moderated by: Dr. Isabel Gimeno

1) AVIAN INFECTIOUS LARYNGOTRACHEITIS: INNOVATIVE SEROLOGICAL FOR DIAGNOSIS, VACCINATION MONITORING AND DIVA TESTING

S. Lesceu*[1], M. Gaimard[1], K. Mouacha[1], C. Redal[1], J-E. Drus[1], C. Lefebvre[1], A. Harmois[1], and P. Pourquier[1]

[1]IDvet, 310 rue Louis Pasteur, 34790 Grabels – FRANCE

Avian infectious laryngotracheitis (ILT) is a respiratory disease of chickens caused by the infectious laryngotracheitis virus called Gallid herpesvirus 1. ILT leads to major losses as a result of mortality and/or decreased egg production. Vaccination is an essential tool for ILT disease control.

Different types of vaccines are commercially available. Conventional live attenuated vaccines (TCO and CEO) based on native virus (partially or totally inactivated) offer good protection but can produce latent infections and reactivation of the virus in the field. Vector vaccines are created by genetic modification(s) of vector microorganisms and the integration into their genomes of exogenous gene(s) encoding for immunogenic protein(s) from viruses responsible of diseases of interest. In the case of poultry vector vaccines, the Fowl Pox Virus (FPV) or the Herpes Virus of Turkey (HVT) are commonly used as vector virus. One or more exogenous genes may be expressed to ensure stronger protection or to widen the spectrum of protection to more diseases. Benefits associated with vector vaccines include biosecurity, protection efficiency, ability to breakthrough passive immunity, and long-lasting immunity. Additionally, the use of vector vaccines allows to implement DIVA strategies (Differentiation between Infected and Vaccinated Animals). In the case of ILT, two types of vaccines exist, one based on the gI protein, and the other on the gB protein.

Given that the conventional serological kits do not efficiently detect seroconversion to vector vaccines, the ID Screen® ILT gB Indirect and the ID Screen® ILT gI Indirect innovative ELISA's were developed to monitor respectively FP-ILTgB and HVT-ILTgI vaccines.

2) MAREK'S DISEASE VIRUS REFERENCE LABORATORY AT THE PIRBRIGHT INSTITUTE, UK

V. Nair[1] and S. Baigent[1]
[1]*Avian Oncogenic Virus Group, The Pirbright Institute, United Kingdom*

The World Organisation for Animal Health (OIE) Marek's Disease Virus Reference Laboratory (https://www.pirbright.ac.uk/diagnostics-surveillance/mareks-disease-virus-reference-laboratory) at The Pirbright Institute, UK (formerly the Institute for Animal Health), was established in the 1980s and received full ISO/IEC 17025 accreditation from the United Kingdom Accreditation Service in April 2018. It is now the world's only accredited Reference Laboratory for Marek's disease virus. Accreditation required extensive validation of the diagnostic tests (in terms of reproducibility, repeatability, sensitivity and specificity), and demonstration of the quality control of documentation, samples, processes, reagents, equipment and personnel. The laboratory undergoes an annual UK Accreditation Service audit to maintain accreditation. This confirms to customers, regulatory authorities and accreditation bodies that the laboratory is competent to perform the tests.

The accredited tests are four real-time PCR assays for specific detection and differentiation between virulent MDV field strains, CVI988 vaccine, HVT vaccine, and MDV-2 vaccine/field strains in DNA extracted from submitted samples of chicken organs, feather tips and poultry house dust. Samples from other MD-susceptible species (turkeys, partridges, pheasants) can also be tested, although tests on these species are not currently accredited. Samples are received, via veterinary surgeons, from commercial flocks, backyard hobby flocks, and zoo birds. Results are semi-quantitative and present the relative level of each virus in the sample. Depending on the customer requirements and the PCR tests requested, the results can be used to confirm a diagnosis of Marek's disease, to investigate suspect Marek's disease, or to monitor the success of vaccination.

3) NUCLEOTIDE IDENTITY OF TWO ILTV HOT-SPOT RECOMBINATION REGIONS FOR DETERMINATION OF ORIGIN AND VIRUS QUANTIFICATION

A. Loyola[1], P. Venegas-Plettenberg[1], H. Hidalgo[2], M. García[3], H. Reyes[1], L. Solarte[1], D. Ivulic[2], N. Gutierrez[1], M. Sandoval[1], and C. A. Loncoman
[1]*Instituto de Bioquímica y Microbiología. Laboratorio Virología. Facultad de Ciencias, Universidad Austral de Chile, 5900000, Chile*
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Gallid alphaherpesvirus-1 (GaHV-1) also known as the Infectious laryngotracheitis virus (ILTV) cause economic losses to the poultry industry due to mild and severe respiratory disease. Live attenuated vaccines are widely used to control disease worldwide. These vaccines are generally effective in controlling clinical disease. However, ILTV vaccine-derived viral subpopulations allowing the rise to vaccine revertant causing outbreaks of the disease. Additionally, both vaccine and field strains undergo recombination generating new highly virulent field strains. Recently, a hot spot recombination region was described within the ILTV genome including the codifying region ICP4. In this work, we sequenced four samples obtained from ILTV outbreaks. From the amplified sequences, we selected two segments from the ICP4 gene (ICP4-1= 509nt and ICP4-2= 661nt) that aligned to the ILTV reference genome NC_006623 ranging from nucleotide 1 to 509 (ICP4-1) and from 3541 to 4202 (ICP4-2). Phylogenetic analysis of the amplified samples indicate high nucleotide similarities with the tissue culture origin (TCO) vaccine strain. We cloned these sequences in order to use these vectors for viral quantification and differentiation based on high resolution melting (HRM) analyses. The high variability of the ICP4 gene serves to classify viral strains by HRM analysis without any further sequencing. HRM of the ICP4 segments was capable to differentiate sequences based on at least nine informative SNPs sites. These results include a molecular characterisation of Chilean field strains and was possible by funding obtained from the: Center for science and global sustainability (Virginia Tech – Universidad Austral de Chile).

4) SIMPLIFYING INFECTIOUS LARYNGOTRACHEITIS SEQEUNCE TYPING FOR CLINICAL APPLICATION

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It is the precise understanding of disease epidemiology, source of infection and transmission patterns that allows for the development of effective prevention, control and eradication methods. Infectious Laryngotracheitis is a contagious Herpes virus that causes upper respiratory disease of chicken leading to significant economic losses in form of mortality and egg production losses. Characterization of Infectious Laryngotracheitis viruses by sequence typing can be achieved by amplifying and sequencing five large genetic segments, which classify the virus into nine genotypes. Given the large size of the target segments, Infectious Laryngotracheitis sequence typing can only be performed from viral isolates or from clinical samples with high viral copy numbers. The purpose of this study is to reduce the number and size of target segments necessary for Infectious Laryngotracheitis sequence typing to allow for sequence typing directly from clinical samples. Three targets were identified, primers were designed to amplify and sequence these targets. Amplification and sequencing was successful from clinical samples with lower copy number. Four of the closely related genotypes merged into two groups. This means that the new assay typed Infectious Laryngotracheitis into seven instead of nine genotypes. In spite of this reduction of discriminatory power, the new assay still preserve the same utility of the nine genotypes assay. Meanwhile, the new assay would allow for the characterization of many more ILT cases. This in turn allows for better understanding of ILT epidemiology and more effective control and eradication efforts.

5) THE DEVELOPMENT OF AGENT-BASED AND MATHEMATICAL MODELS FOR MAREK'S DISEASE VIRUS (MDV) LYTIC AND LATENT INFECTIONS

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Marek's disease virus (MDV) is an acute-transforming *Alphaherpesvirus* of chickens that causes paralysis and T-cell lymphoma formation. Despite the ability of vaccines to reduce the impact of Marek's disease in poultry production, vaccinated chickens support superinfection and transmission of virulent field strains. Considering that MDV ecology has been manipulated in poultry production by the concentration of genetically-uniform, imperfectly-vaccinated hosts of ever-decreasing lifespan; the classic model of MDV's early lytic phase, latency, and reactivation, appear to have been under selective pressures. To aid in our understanding of this evolution of virulence, we are developing agent-based (NetLogo) and mathematical models for MDV infection of the spleen, a major target organ for *in vivo* lytic replication and latency establishment. With NetLogo, we hope to capture a realistic spatial simulation of MDV entry into the spleen and transmission among cell types, including differences in susceptibility to infection and the impact of immune responses to virus replication over time. In addition to this agent-based simulation, we are also developing differential equation-based models for these cell-to-cell interactions during lytic infection, and during reactivation from latency. We are using recombinant viruses (pRB-1B, GFP-2a-Meq, UL49-2a-RFP) and pRB-1B, ICP27-2a-GFP, UL42-2a-E2-Crimson, and UL49-2a-RFP), that have fluorescent proteins defining latency/lytic infections and immediate early, early, and late stages of infection, respectively, to help inform these models. Once established for a basic MDV-infection, we plan to run simulations based on observations from different pathotype MDVs to help discern evolutionary selection pressures that have contributed to the increased virulence of MDV field strains.

Session 1: Epidemiology and Diagnosis Panel 2

Moderated by: Dr. John Dunn

1) MAREK'S DISEASE IN ITALIAN BROILER BREEDERS: EVIDENCES THAT INCREASINGLY STRINGENT CLEANING AND DISINFECTION PROCEDURES CAN REDUCE THE VIRAL ENVIRONMENTAL LOAD

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Despite the vaccination, usually applied *in ovo* and repeated on day of hatch, Marek's disease (MD)-associated losses could be relevant for Italian broiler breeders. The present study was designed to monitor *Gallid alphaherpesvirus 2* (GaHV-2) in dust and feathers throughout four subsequent production cycles of Italian broiler breeders, vaccinated with HVT + CVI988, experiencing MD-associated mortality during the laying period. Samples were collected during the pullet-raising phases and GaHV-2 was detected by a newly developed nested PCR protocol optimized for the amplification of the entire *meq* gene from dust and feathers. As the *meq* gene carries molecular markers for field and vaccine GaHV-2 strains characterization and differentiation, sequence analysis was performed. Moreover, birds were tested for genetic susceptibility or resistance against MD by a PCR protocol targeting the *B-LβII* alleles associated with five most common B haplotypes: B2, B13, B15, B19 and B21. Field GaHV-2s were consistently detected in the monitored cycles, despite the progressive reinforcement of biosecurity, cleaning and disinfection procedures in the farm. However, in the last two cycles, detection of virus from feathers and dust revealed a delayed infection of birds and the co-existence of field and vaccine genomes in dust suggesting a reduction of field virus load. Results showed that field viruses circulate despite vaccination and that strict application of biosecurity and proper cleaning and disinfection can reduce field GaHV-2 environmental load and delay the infection. *B-LβII* alleles responsible either for susceptibility or resistance to MD were found in sampled birds, suggesting heterogeneous genetic profile of the population.

2) AN EPIDEMIOLOGICAL SURVEY IN VACCINATED ITALIAN BROILER FLOCKS REVEALS THE CIRCULATION OF GALLID ALPHAHERPESVIRUS 2 STRAINS WITH MOLECULAR CARACTERISTICS OF HIGH VIRULENCE

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Losses due to Marek's disease (MD) in Italian broiler chickens have been significantly reduced since the introduction of vaccination and, when they occur, are mainly associated to clinical disease and condemnation at slaughter. Vaccination protocols consist of vaccination at the hatchery with the Herpesvirus of turkey (HVT) or with an association of HVT+Rispens. In 2018 outbreaks of MD were reported in HVT+Rispens-vaccinated organically reared broilers, bringing back the attention to MD in broilers. In order to investigate the circulation of the *Gallid alphaherpesvirus 2* (GaHV-2) in conventional and organically reared broiler birds, an epidemiological survey was conducted on 40 flocks housed in 22 farms located throughout Italy. The virus was detected from environmental dust samples, collected from fan louvers at the end of the productive cycle, by a nested PCR protocol able to amplify the entire *meq* gene. Amplicons were molecularly characterized by sequencing and sequence analysis. Twenty-eight out fourty flocks resulted to be positive for GaHV-2, which in most cases (68%), at sequence analysis, were found to be of vaccine origin. However, in nine flocks, field viruses with molecular characteristics of high virulence were detected. The circulation of high virulence GaHV-2 strains in the Italian broiler compartment, both conventional and organic, confirms that vaccination is essential to guarantee the protection against MD in broilers. This is especially true for organically reared birds in which the rearing period is extended up to 81 days of age, and the use of conventional detergents and disinfectants is restricted.

3) MAREK'S DISEASE VIRUS SEROTYPE 2 CIRCULATES NATURALLY IN COMMERCIAL POULTRY FLOCKS: A SURVEY USING REAL-TIME PCR AND LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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Marek's disease virus serotype 2 (MDV-2, *Gallid herpes virus 3*) strains infect chickens, but are non-oncogenic, and are considered to be only mildly- or non-pathogenic. The MDV-2 strain SB-1 was introduced as a vaccine virus in the 1980s, administered in combination with herpesvirus of turkeys in a bivalent live-attenuated vaccine against Marek's disease. The SB-1/HVT bivalent vaccine effectively protects against very virulent MDV field strains, but is less efficient against very virulent-plus strains. It is used in layer, broiler and breeder chickens in North America and Asia, but is not used in Europe.

A selection of samples from those submitted to the Pirbright Institute's Marek's Disease Virus Reference Laboratory between 2015 and 2020 was tested by MDV-2-specific real-time PCR. This snapshot of samples showed that MDV-2 is prevalent in many layer, broiler, and breeder chicken flocks (but not turkey flocks), being detected in tissue samples from the chickens and in dust collected from the chicken houses. MDV-2 can be detected in either the presence or absence of virulent MDV field strains, CVI988 and HVT vaccines, and in healthy flocks as well as those with clinical signs of Marek's disease. This shows that MDV-2 strains circulate freely and naturally in chicken flocks, but may not offer protection against Marek's disease.
To complement real-time PCR, an MDV-2-specific loop-mediated isothermal amplification test, with 3-stripe lateral flow device read-out, was developed as a novel, simple test for MDV-2 for rapid sample-to-result testing in the field.

4) CONCURRENT INFECTION OF MAREK'S DISEASE WITH OTHER PATHOGENS IN COMMERCIAL POULTRY FARMS, PLATEAU STATE, NIGERIA

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Most Marek's disease (MD) clinical diagnoses are based on history, signs, gross and histo-pathological lesions. The intensive rearing methods of poultry are responsible for the marked increase commercialization of poultry industry. Most areas of Nigeria have seen outbreaks of co-infected poultry diseases as immunosuppression, which cause huge economic losses to the industry. However, couple with standard of living and management problems, there are no permanent measures to combat the challenges the farmers are facing. This study presents the poultry diseases diagnosed in ECWA Veterinary Clinic in 2017 with MD co-infected with concurrent secondary infections. Total clinical cases presented from January to December were 4125 out of which were 446 MD cases (11%), 749 Fowl Typhoid (18%), Coccidiosis 838 (20%), Newcastle Disease 290 (7%), E.coli 296 (7%), Colibacillosis 241 (6%), Chronic Respiratory Disease 289 (7%), Infectious Bursa Disease 234 (6%), and the rest were less than 5%. Co-infection MD, has Newcastle Disease 18, Management Problems and Coccidiosis 2 while Fowl Typhoid was 1. This study advocates further studies in multiple disease interaction since avian systems could host a wide range of commensal and potential pathogenic bacteria or viruses that might interact with each other. Such interactions could be either synergistic or antagonistic, which determines the severity of the disease complex and can lead to improved intervention strategies aimed at controlling disease spread.

5) EVIDENCE OF HISTORICAL RECOMBINATION IN THE INFECTIOUS LARYNGOTRACHEITIS VIRUS (ILTV) STRAINS CIRCULATING IN CANADA

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Infectious laryngotracheitis (ILT) caused by *Gallid herpesvirus-1* (GaHV-1) or infectious laryngotracheitis virus (ILTV) occurs sporadically in poultry flocks in Canada. Live attenuated vaccines are being used routinely to prevent and control outbreaks of ILT. Recombination events have occurred between vaccine and wild-type strains of ILTV leading to emergence of more virulent strains. In this study, five Canadian ILTV isolates linked to ILT outbreaks in two provinces in Eastern Canada (Ontario and Quebec) were whole genome sequenced. These 5 whole genome sequences of ILTV were subjected to phylogenetic analysis along with publicly available 50 ILTV complete genome sequences, including 19 Canadian sequences. It was noticed that 2 Canadian ILTV strains recovered from Ontario were clustered with CEO vaccine strain, Poulvac-LT vaccine. Whereas the other 3 ILTV strains isolated in Quebec were categorized either as a CEO revertant strain or wild-type strain. Additionally, multiple software programs were used to find the evidence of potential recombination events. Recombination analysis revealed that one of the Ontario ILTV sequences and one of the Quebec ILTV sequences were potential parenteral strains for a Korean origin isolate (KO-30678/14) and a United States origin ILTV isolate (US-1874C5) respectively. Interestingly, recombination network analysis of ILTV sequences revealed clear evidence of historical recombination in the ILTV strains circulating in Canada. Our findings highlight the importance of natural recombination process in the evolution of ILTV in Canada.

Session 1: Immunology Panel 1

Moderated by: Dr. Shayan Sharif

1) CHARACTERIZATION OF THE ROLE OF GAMMA DELTA T CELLS IN MAREK'S DISEASE VIRUS VACCINATED AND INFECTED CHICKENS

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Marek's disease vaccines reduce the incidence of MD but cannot control virus shedding. Therefore, it is crucial to develop new vaccines and elucidate mechanisms of immunity to Marek's disease virus (MDV). Among the innate responses against MDV, gamma delta (γδ) T cells may play a significant role as a first line of defense due to their high frequency in chicken blood and tissues. We hypothesized that MDV interacts with γδ T cells and that these cells play a role in immunity against MD. To investigate this hypothesis, γδ T cells were obtained from MDV vaccinated and challenged chickens and the expression of cytokines and related molecules was analyzed using flow cytometry. Here we demonstrated that administration of MDV vaccine induced interferon (IFN)-γ^+CD8$^+$ γδ T cells at 4 days post-infection (dpi) and transforming growth factor (TGF)-β^+ γδ T cells in lungs at 21 dpi. γδ T cells from MDV-challenged chickens exhibited cytotoxic activity as shown by an increase in frequency of CD107$^+$ cells without ex vivo stimulation. Maximum stimulation of cytotoxic γδ T cells was observed in the vaccinated/challenged group following ex vivo stimulation of the cells with phorbol 12-myristate 13-acetate (PMA)/ionomycin. These results suggested that MDV vaccines induce the activation of effector γδ T cells which may be involved in the development of protective immune responses against MD in young chickens.

2) THE ROLE OF CHICKEN IFNS AND NK CELLS IN MAREK'S DISEASE VIRUS INFECTIONS

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Marek´s disease virus (MDV) is an alphaherpesvirus that causes deadly lymphomas in chickens. MDV vaccines protect animals from clinical disease but do not provide sterilizing immunity, allowing field strains to circulate and evolve in vaccinated flocks. To prevent this, we need a better understanding of innate and adaptive immune responses against MDV. In this study, we assessed the role of chicken IFNs and NK cells in MDV infections. We analyzed the strong antiviral effect of IFNα and IFNγ against MDV in vitro and demonstrated that both cytokines can delay disease onset and progression in vivo. In turn, blocking endogenous IFNα with monoclonal antibodies accelerated disease. IFNs efficiently activate NK cells that can kill virus-infected cells and are crucial for the induction of adaptive immune responses. We could demonstrate that very virulent RB-1B MDV and the live-attenuated CVI988 vaccine can efficiently infect primary NK cells and alter their activation. Flow cytometry analysis revealed that both RB-1B and CVI988 enhance NK cell degranulation and increase IFNγ production in vitro. In addition, we could show that the major MDV-encoded oncogene meq contributes to NK cell activation using knockout viruses. Taken together, our data reveal the effects of IFNα and IFNγ on MDV infections and improve our understanding of innate immune responses against MDV. Moreover, we could demonstrate that MDV efficiently infects NK cells in vitro, enhances their degranulation and increased IFNγ production.

3) MECHANISM OF MAREK'S DISEASE VACCINE-MEDIATED IMMUNITY

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The antiviral activity of vaccine-induced immunity that markedly reduces the level of early cytolytic infection, production of cell-free infectious virus particles in the feather follicle epithelial cells (FFE), and lymphoma formation by interrupting the normal cascade of pathogenic events, is a significant factor in protective efficacy of Marek's disease (MD) vaccines. Despite the success and availability of several vaccines that have greatly reduced the losses from MD, the molecular mechanism of vaccine-induced immunity is poorly understood. To provide insight into possible role of adaptive immune system in vaccine-mediated protection, we bursectomized birds at day of hatch and vaccinated them eight days later after confirming the absence of circulating B cells in blood samples. The birds were challenged one-week post vaccination with or without receiving adoptive lymphocytes from age-matched control birds. The study also included vaccinated/challenged and non-vaccinated/challenged groups as controls. The non-vaccinated/challenged birds exhibited MD symptoms and eventually developed tumors. The bursectomized/vaccinated and challenged groups, with or without adoptive lymphocyte transfer, were fully protected without any clinical symptoms, nerve enlargement or development of T cell lymphomas. Immunohistochemical analysis of skin tissues at termination revealed a significant number of virus particles produced in the FFE of the non-vaccinated/challenged birds. No virus particle was detected in the FFE of the vaccinated/challenged birds. From the bursectomized, vaccinated/challenged groups, only a few replicating viruses were detected in the skin of birds that received adoptive lymphocytes prior to challenge. The study shows that B cells do not play any role in MD vaccine-mediated immunity.

4) *IN VITRO* INTERACTIONS OF CHICKEN PROGRAMMED CELL DEATH 1 (PD-1) AND PD-1 LIGAND-1 (PD-L1)

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Immune T cell exhaustion is a state of ineffective T cell response that occurs during chronic (latent) viral infections and cancer. PD-1 and PD-1/PD-L1 pathways play a key role in the T cell exhaustion. Recent studies have shown that PD1 is highly expressed on exhausted T cells, and PD-1/PD-L1 checkpoints are targets of immunotherapy. Currently, in humans, usage of monoclonal antibodies as blockades of PD-1 and PD-L1 pathways, and inhibitors of the PD-1/PD-L1 interaction are gaining momentum. Thus, we have determined the *in vitro* characteristics and binding interactions of chicken PD-1 (chPD-1) and PD-L1 (chPD-L1) and developed a panel of specific monoclonal antibodies against the two proteins. ChPD-1 and chPD-L1 sequence identities and similarities were lower compared with those of humans and other mammalian species. Based on the three dimensional (3D) structural homology, chPD-1 and chPD-L1 were similar to 3D-structures of mammalian PD-1 and PD-L1. Further, Ig V domain of chPD-1 and the Ig V and Ig C domains of chPD-L1 were highly conserved with the mammalian counterparts. *In vitro* binding interaction studies using Superparamagnetic-Dynabeads® confirmed that recombinant soluble chPD-1/PD-L1 fusion proteins and surface chPD-1/PD-L1 proteins interacted with each other on COS cells. Two monoclonal antibodies specific against chPD-1 and five antibodies against chPD-L1 were developed and confirmed by immunofluorescence staining and Western blotting. The results of this work will form the technical basis for future research to explore the role of PD-1/PD-L1 pathway in the latency mechanisms and immunosuppression of Marek's disease and other chronic viral infections of chickens.

5) DURATION OF IMMUNITY ELICITED BY A VECTOR HVT-H5 VACCINE IN COMMERCIAL LAYERS

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Biosecurity, monitoring, stamping out and compensation are the key tools to control outbreaks of highly pathogenic avian influenza (HPAI). However, in countries where the disease became endemic vaccination is a useful companion tool to mitigate the losses. Long duration of immunity is usually achieved by repeated administration of killed vaccines.
HVT vector vaccines (rHVT) have several beneficial characteristics, including their safety, genetic stability and long-lasting persistence in the birds, which results in long duration of immunity as already demonstrated for a rHVT-ND vaccine against velogenic Newcastle disease virus (NDV) challenges up to 72 weeks of age (Palya *et al.,* 2014).

The presented experiment aimed at assessing the duration of the elicited immunity by a rHVT-H5 vaccine against AIV H5 (Vectormune® AI) up to 100 weeks of age to cover the increased lifetime of new layer genetic lines. Commercial layers provided artificially with AIV H5 antibodies were vaccinated subcutaneously at day-old with rHVT-H5 and Rispens vaccines in combination. Vaccine virus presence was checked in spleen, humoral immune response to the insert was monitored during the laying period.
Humoral antibody level against AIV H5 remained stable up to 100 weeks of age, persistence of rHVT-AI virus in spleen could be demonstrated at 100 weeks of age.
The results of this experiment provided further evidence on the long duration of immunity elicited by a rHVT vaccine against AIV(H5) when applied in combination with Rispens vaccine to commercial layers, in the presence of maternal antibodies to the targeted disease.

Session 1: Immunology Panel 2

Moderated by: Masahiro Niikura

1) ASSOCIATION OF THE MAGNITUDE AND FUNCTIONAL ABILITIES OF T CELL RESPONSES TO MAREK'S DISEASE VIRUS AND DISEASE RESISTANCE

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Both oncogenic and attenuated vaccine strains of Marek's disease virus (MDV) are cell-associated and thus it has been postulated that cell-mediated immunity plays a crucial role in immunity against the disease. Here, we examined effector and memory T cell responses to two immunogenic MDV antigens (PP38 and Meq) in the MHC defined genetically resistant (B21 haplotype) and susceptible (B19 haplotype) chicken lines using both cellular and molecular immunological techniques including T cell epitope mapping. Immuno-dominant and subdominant T cell epitopes within PP38 and Meq of MDV in both B21 and B19 haplotypes were identified using chicken IFN-gamma ELISPOT assay. The results demonstrated that effector and memory PP38 or Meq-specific T cell responses were dominated by IFN-gamma producing TCRVbeta1$^+$ CD4$^+$ T cells which also express IL-2, IL-4 and IL-10 and proliferate in vitro in an antigen-specific manner. Interestingly, the frequencies and functional abilities of anti-MDV T cells were more pronounced in MDV infected or vaccinated B21 haplotype chickens compared to the susceptible line. Taken together, the results may explain the disease resistance in B21 haplotypes and provide some information on correlation of protection against Marek's disease.

2) EFFECT OF DOSE AND STRAIN WITH IN OVO VACCINATION OF HERPESVIRUS OF TURKEY ON THE INNATE AND ADAPTIVE IMMUNE RESPONSES OF COMMERCIAL MEAT-TYPE CHICKENS

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In ovo vaccination with herpesvirus of turkey (HVT) enhances innate and adaptive immunity in Specific-Pathogen-Free Avian Supplies (SPAFAS) chickens. This study evaluated the ability of HVT to accelerate immunocompetence in meat-type chickens when administered *in ovo* at 18ED and optimized the conditions (dose and strain) to achieve the maximal benefit. A conventional HVT vaccine was given at recommended dose (RD), HVT-RD=6,080 plaque forming units (PFU), double-dose (2X), half-dose (1/2), or quarter-dose (1/4). Two recombinant HVTs (rHVTs) were given at RD: rHVT-A=7,380 PFU, rHVT-B=8,993 PFU. Most, if not all, treatments enhanced lymphoproliferation with Concanavalin A and increased the percentage of granulocytes at day of age. Dose had an effect and HVT-RD was ideal. An increase of wing-web thickness to phytohemagglutinin-L was detected with HVT-RD only. An increased percentage of T cells (CD3+, CD4+) and increased expression of MHC-II+ in most cell subsets (CD45+, non T, CD3+, CD8+, TCR γδ) was observed with HVT-RD; HVT-1/2 and rHVT-B shared some similar features with the latter. Expression of MHC-I+ was decreased in most cell phenotypes with HVT-RD, HVT-2X, and rHVT-A. The effect of *in ovo* HVT on humoral immune responses (antibody responses to KLH and to a live infectious bronchitis/Newcastle disease vaccine) was minimal. Further evaluation of IFN, IFN receptors and TLR in chickens that received HVT-RD showed an increase of TLR-3 and IFN-γR2 transcripts in the spleens. Our study demonstrates *in ovo* vaccination with HVT in meat-type chickens can accelerate innate and adaptive immunity; we can optimize such effect by modifying vaccine dose.

3) TRANSCRIPTOMIC PROFILES AND DIFFERENTIAL GENE EXPRESSION OF SPLENIC CD4+ T CELLS IN RESPONSE TO VACCINATION AGAINST MAREK'S DISEASE

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Marek's disease (MD) is a contagious viral neoplastic disease of chicken, which is characterized with T-cell lymphoma. Vaccination has been employed as the primary control of MD since the 1970s. MD vaccine efficacy, however, varies attributable to factors including chicken genetics. To elucidate genomic mechanism underlying vaccine efficacy, this study was designed to probe the transcriptomic differences between two highly inbred lines of White Leghorns that exhibited striking difference in MD incidence in response to HVT vaccination followed by MDV challenge. The two inbred lines, line 6_3 and line 7_2, share a common MHC *B*2*-haplotype. Spleen tissues were sampled from birds 10-days post inoculation of 2,000 PFU HVT or CVI988/Rispens and used in isolation of CD4+ T cells for total RNAs. The total RNA samples were subjected to deep sequencing on an Illumina NextSeq sequencer. All pass-filter reads were subjected to mapping, quantification and differential expression tests followed by functional profiling. A total of 12,024 genes/transcripts was identified. CVI988/Rispens and HVT induced 39 and 57 differentially expressed genes in line 6_3 birds, 1,337 and 80 genes in line 7_2 birds, respectively. Differentially expressed genes in response to CVI988/Rispens were enriched in 20 and 695 biological function terms, and in HVT, in 83 and 67 functional terms for lines 6_3 and 7_2, respectively, which included biological term of defense response; reactome pathways of innate immune system, immune system, and advanced glycosylation endproduct receptor signaling; and transcriptional factor PU.1, which functions as a major transcriptional activator of tumor suppressor gene.

4) PHENOTYPIC CHANGES IN SPLENOCYTES FOLLOWING INFECTION WITH A VERY VIRULENT PLUS MDV IN COMMERCIAL MEAT TYPE CHICKENS

I.M. Gimeno, T. Kaeser, A.L. Cortes, B.A. López de Juan Abad, and A. Boone
North Carolina State University

Very virulent plus Marek's disease virus (vv+MDV) can induce severe immunosuppression in commercial meat type chickens. In a previous work, we have demonstrated that 30 days after infection (dpi) with vv+MDV there was a severe destruction of lymphocytes in the spleen. The objective of the present study was to evaluate chronologically the phenotypic changes in the splenocytes after infection with vv+MDV strain 686. Commercial meat type chickens were challenged by contact at day of age or remained unchallenged as the negative control group. At 6, 20, and 30 dpi spleens were collected to evaluate cell phenotype by flow cytometry. At 6 dpi, the percentage of B cells and TCRgd T cells in the infected chickens was reduced but the percentage of TCRab T cells, CD4+CD8b$^+$, and CD4$^+$CD8b$^-$ was increased. At 20 dpi, frequencies of B cells and TCRgd T cells were also reduced. In addition, the percentage of dead cells in the TCRab T cells, CD4$^+$CD8b$^+$, CD4$^+$CD8b$^-$, CD4$^-$CD8b$^-$, and CTLs populations increased. At 30 dpi, the percentage of CD45$^+$, B cells, CTLs, TCRgd T cells, and CD4$^+$CD8b$^-$ was reduced. Furthermore, the percentage of dead cells of CD45$^+$, T cells, TCRab T cells, CD4$^+$CD8b$^-$, CD4$^-$CD8$^-$, and CTL was higher than in the control group. MCH-I expression was increased in T cells at 6, 20, and 30 dpi. MHC-II expression was decreased in all subsets of T cells at 6 and 20 dpi but increased at 30 dpi. The significance of these results on the pathogenesis of MDV-induced immunosuppression will be discussed.

AS/AU Panel 1: Related Virus-Factors. Sponsored by: Boehringer Ingelheim

Moderated by: Dr. Stephen Walkden-Brown and Dr. Aijian Qin

1) MOLECULAR APPROACHES TO LIMIT HERPESVIRUS RECOMBINATION AND IMPROVE VACCINE SAFETY

Parisa Armat[1], Paola K. Vaz[1], Carol A. Hartley[1], Glenn F. Browning[1], Amir H. Noormohammadi[1], and Joanne M. Devlin[1]
[1]Asia-Pacific Centre for Animal Health, Department of Veterinary Biosciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Victoria, Australia

Herpesviruses UL12 encodes a recombinase protein that is conserved among all members of the *Herpesviridae* family. UL12 facilitates recombination during replication in HSV-1 and it also has a role for DNA repair through single-strand annealing and exonuclease activity in HSV-1 and Marek's disease virus. The role of UL12 in infectious laryngotracheitis (ILTV) has not been characterised.

This study is manipulating the genome of ILTV by replacing the UL12 gene with codon de-optimized versions of the gene in order to reduce the translation efficiency while conserving the amino acid sequence. Mutants viruses with altered ORFs are identified by the presence of green fluorescent protein, and viruses are passaged on chicken cell cultures, with final purification and isolation of these viruses confirmed by PCR and sequencing. Further *in vitro* and *in vivo* studies are required to determine the effects of these changes on the rate of recombination of these recombinase virus mutants.
The result of this study will help to characterise the role of the ILTV UL12 gene and help to establish a methodology to characterise other essential or extremely important genes in the ILTV genome. As the UL12 gene in ILTV is likely to play a role in viral recombination, mutants developed in this project may have a reduced capacity to recombine with other viruses and may be candidates for new and safer ILTV vaccines.

2) EXPRESSION LEVEL AND FUNCTION ANALYSIS OF P53-RELATED LONG NON-CODING RNAS IN SPLEEN TISSUES OF MAREK'S DISEASE VIRUS-INFECTED CHICKENS

S. Han[1], T.G. Liu[1], C. Li[1], X.X. Tan[1], Y.Q. Liu[1], L.M. Han[1], and L.P. Han[2,*]
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Chicken Marek's disease (MD) is a highly infectious lymphoproliferative disease caused by Marek's disease virus (MDV) and endangers the healthy development of chicken industry. Long non-coding RNAs(lncRNAs) may be involved in the occurrence of MD.

In this study, the spleen tissues of SPF chicks were collected 1, 7, 14, 21, 35, and 42 days post infection with MDV BJ-1 strain, and qRT-PCR was used to detect the relative mRNA expression of p53 and 6 p53-related lncRNAs (lncRNA-53BP1-1, lncRNA-53BP1-2, lncRNA-53BP1-3, lncRNA-53BP2, lncRNA-TP53TG5, lncRNA-TP53I3) predicted by RNA-seq, and Western Blot was used to detect the relative expression of p53 protein; overexpression vectors pEGFP-N1-lncRNA-53BP1-2, pEGFP-N1-lncRNA-53BP1-3 and shRNA interference vectors pGreen-lncRNA-53BP1-2, pGreen-lncRNA-53BP1-3 were constructed. After these vectors were transfected into the chicken fibroblast cell line DF-1, the expression levels of lncRNA-53BP1-2, lncRNA-53BP1-3 and p53 in DF-1 cells were analyzed.

The results showed that with the extension of MDV infection time, the transcription level of lncRNA-TP53I3 in chicken spleen tissue showed a trend of up-regulation first and then down-regulation, while the other five p53-related lncRNAs were the opposite; MDV infection leads to an up-regulation of p53 protein expression in chicken spleen tissue; overexpression or knockdown of lncRNA-53BP1-2 and lncRNA-53BP1-3 can lead to up-regulation or down-regulation of p53 expression in DF-1 cells.
In summary, lncRNA-53BP1-2 and lncRNA-53BP1-3 may participate in the regulation of the p53 pathway, and then function as important regulatory factors in the pathogenesis of MDV, but their correlation and specific regulatory mechanisms need to be further verified and explored.

3) THE DEAD-BOX HELICASE 5 INVOLVED IN AVIAN ONCOGENIC HERPESVIRUS INFECTION BY AFFECTING IFN BETA SIGNALING

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The DEAD-box helicase 5 as an ATP dependent RNA helicase, functions as a transcriptional co-activator in tumorigenesis and regulates viral replication in several viruses. It is believed that it is mainly involved in cellular RNA metabolism regulation of lymphoma or viral infection. Avian oncogenic herpesvirus Marek's disease virus causes malignant lymphomas in chickens. However, the exact mechanism of innate host responses to MDV infection still remains unclear. In this study, we presented that MDV inhibited the production of interferon beta (IFN-β) in chicken embryo fibroblasts via increasing the expression and promoting the nuclear aggregation of DDX5. The DDX5 blocked MDA5/TLR3 signaling by activating the extracellular regulated protein kinases which were negatively regulated by a fundamental transcription factor, interferon regulatory factor 1. The absence of DDX5 distinctly diminished the inhibition of MDV to IFN-β production, resulting in the reduction of MDV replication in chicken embryo fibroblasts. Taken together, our investigations demonstrate that MDV inhibits IFN-β response by targeting DDX5-mediated signaling to facilitate viral replication.

4) FULL GENOMIC CHARACTERISATION OF AN EMERGING "VACCINE-LIKE" INFECTIOUS LARYNGOTRACHEITIS VIRUS IN AUSTRALIA REVEALED ITS TRUE IDENTITY

Ahmad J. Sabir[1], Olusola M. Olaogun[1], Denise O'Rourke[1], Mauricio J.C. Coppo[1], Joanne M. Devlin[1], Barbara Konsak-Ilievski[1], and Amir H. Noormohammadi[1]
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Infectious laryngotracheitis virus (ILTV) is an alphaherpesvirus that infects chickens, causing upper respiratory tract illness and substantial economic losses to the commercial poultry industry worldwide. Due to its geographical isolation, Australia has had a unique population of ILTV genotypes, and this has provided the researchers with an excellent opportunity to examine the evolution of herpesviruses. Recent studies on the evolution of ILTV have reported the emergence of recombinant ILTVs in Australian poultry flocks. More recently, there has been an increasing number of field outbreaks caused by ILTV isolates that are indistinguishable from serva vaccine strain using current molecular tests that rely on restriction fragment analysis of selected regions of the viral genome. In this study, whole-genome analysis of one of the field isolates revealed a new class of ILTV, identified here as class 7b, emerged as a result of recombination probably between another recombinant strain and the Serva vaccine strain (now reclassified as 7a). Interestingly, the 7b virus had the highest similarity to class 9, a virus that dominates the ILTV population in Victoria, where 7b has never been reported to date. Also, sequence analysis detected sequences unique to class 10, another recombinant virus that became predominant in some states of Australia between 2013 and 2014 but disappeared since then. These results demonstrate the influence of recombination as a continuous process towards more virulent and transmissible ILTVs.

5) CELLULAR IMMUNE RESPONSES ELICITED BY CVI988/RISPENS VACCINE AGAINST MAREK'S DISEASE IN CHICKENS

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Marek's disease virus (MDV) is a highly oncogenic alphaherpesvirus that causes deadly T-cell lymphomas and serves as a natural virus-induced tumor model in chickens. Although MD is well controlled by current vaccines, the mechanisms leading to protective immunity following vaccination are not fully understood. Here, employing multi-parameter flow cytometry, we performed a comprehensive analysis of cellular immunity to the most efficacious MD vaccine, CVI988/Rispens (CVI988), in chickens. We found that CVI988 vaccination elicited significant and long-lasting increase in KUL01$^+$ monocytes/macrophages while B cells decreased in lung, spleen and blood in term of percentage and number. Furthermore, CVI988 vaccination induced significant expansion of γδ T cells and CD8α$^+$ T cells but not CD4$^+$ T cells at early time-points. Such cellular immune responses were not observed in MDV RB1B-infected or HVT-vaccinated chickens. Phenotypic analysis showed that CVI988 vaccination elicited preferential proliferation of CD8α$^+$ γδ T cells and CD8αα co-receptor expression was upregulated on γδ T cells and CD8α$^+$ T cells after immunization. In addition, cell sorting and quantitative RT-PCR showed that CVI988-activated γδ T cells and CD8α$^+$ T cells exhibited differential expression profiles of cytotoxic and T cell-related cytokines. Lastly, we demonstrated that although the vaccine viruses persist in chickens, CVI988 induced memory CD8$^+$ T cells but not γδ T cells. These results have filled a few of gaps in our understanding of cellular immunity to MD and are informative and valuable for vaccine development against oncogenic MDV.

Live Keynote Speaker: Dr. Damania. Sponsored by: Merck/MSD

Boshamer Distinguished Professor & Vice President for Research, University of North Carolina at Chapel Hill School of Medicine

Moderated by: Dr. Karel (Ton) Schat

Dr. Blossom Damania is the Boshamer Distinguished Professor and Vice Dean for Research at the University of North Carolina-Chapel Hill. Dr. Damania's research focuses on oncogenic human herpesviruses and host-pathogen interactions, with a focus on Kaposi's sarcoma-associated herpesvirus (KSHV). She uses a multi-faceted approach towards understanding host-pathogen interactions, host innate immune responses to viral infection, as well as viral oncogenesis.

MODULATION OF CELL SIGNALING PATHWAYS BY KSHV

The presentation will focus on how Kaposi's sarcoma-associated herpesvirus (KSHV) modulates host cell signaling pathways. KSHV is associated with three cancers in the human host including Kaposi's sarcoma (KS), primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). The role of a KSHV viral kinase as well as the contribution of host cellular kinases to lymphomagenesis will be discussed.

Live Industry Speaker: Dr. Guillermo Zavala

Avian Health International LLC, University of Georgia Adjunct Professor

Moderated by: Dr. Maricarmen Garcia

Dr. Zavala is currently the owner and founder at Avian Health International LLC, a poultry consulting business doing veterinary work in over 50 countries. He remains an adjunct professor at UGA, where he collaborates in teaching avian virology, poultry diseases and poultry husbandry. Dr. Zavala has experience from a variety of different experiences such as working at 2 vaccine companies, one primary breeder company, one diagnostic laboratory, and spending 11 years as faculty at UGA doing research on viral pathogenesis, clinical extension and teaching.

CURRENT STATUS OF MAREK'S DISEASE AND INFECTIOUS LARYNGOTRACHEITIS AND STRATEGIES FOR CONTROL

Guillermo Zavala
Avian Health International, LLC, Flowery Branch, Georgia, 30542 U.S.A.

Marek's disease (MD) and infectious laryngotracheitis (ILT) represent two economically important infectious diseases caused by avian herpesviruses. MD is caused by a Gallid Herpesvirus 2 (GaHV-2 or MDV), whereas ILT occurs after infection with a GaHV-1, unrelated to GaHV-2. Infection with MDV in the absence of clinical disease is common even in properly immunized chickens, making it necessary to define MD as a neoplastic and/or immunosuppressive disease with specific features, instead of only an infection with MDV. Chickens affected clinically with MD may develop B cell lymphomas and immunosuppression after infection causing cytolytic replication in B cells and latent infection in T cells. Latently infected T cells may be transformed resulting in neoplasia and paralysis due to infiltration with lymphocytes in the peripheral nerves and the brain. Economic losses in broiler production are derived primarily from condemnations of processed chickens due to "skin leukosis" and occasional neoplastic transformation in the spleen, liver and kidneys. Young broilers rarely express neoplastic or even inflammatory changes associated with MD. However, older broilers and long-lived chickens such as broiler breeders, layer breeders and commercial egg layers with MD express increased mortality, B cell lymphomas and evidence of immunosuppression. Losses associated with mortality in broiler breeders result in increased pullet cost, fertile egg production cost and chick cost. Increased mortality in commercial layers results in augmentation of pullet cost, increased mortality, reduced eggs produced per hen and overall increased egg production cost. Ever since the advent of herpesvirus of turkey- (HVT) based vaccines (serotype 3), followed by serotype 2 vaccines (SB-1 and 301/B1) and finally by serotype 1 vaccines (CVI/988, Rispens) in North America, the poultry industry has been able to gradually increase its response to emerging field viruses with increasing expanded pathogenicity. The CVI/988 Rispens vaccine strain has been long considered the most potent

vaccine strain against MD available in many countries. CVI/988 has been successfully used in the European Union since the early 1970s, albeit it did not become available in the United States until the mid 1990s. Currently, most long-lived chickens are successfully vaccinated with either CVI/988 or CVI/988+HVT vaccines in ovo and/or at hatch. Recently, a new generation of chimeric serotype 1 MD vaccine has been introduced in the United States market, where several million broiler breeders have received such vaccine. Countries commercializing mostly or exclusively processed chickens and with a strict federal inspection system at processing plants utilize MD vaccines in broiler chickens to maintain "leukosis" condemnations at a minimum. MD vaccination is mandatory in all types of chickens some countries, including Brazil. In contrast, many countries or individual broiler integrators distributed in various continents do not vaccinate broilers against MD, especially in areas where broiler chickens are not readily processed but sold live, or simply in countries where MD in broilers is not a concern. MD appears to be under control in most regions of the world, albeit there are infrequent field reports of MD, usually resulting from deficiencies in vaccine transportation, storage, reconstitution or application, as well as deficiencies in biosecurity and husbandry or improper control of immunosuppressive disease. A recent concerning trend in some production units of the US broiler industry has been to temporarily interrupt in ovo MD vaccination as an attempt to reduce 7-day mortality associated with bacterial contamination introduced through in ovo vaccination in eggs or poor microbiological quality after the ban of antimicrobial drug use in hatcheries.

ILT is a respiratory disease involving primarily the upper respiratory tract, including the conjunctiva, nasal passages, larynx, trachea and extrapulmonary bronchi. Severe infections involve less commonly the primary and secondary bronchi, and focal areas of the lungs. ILT is not a respiratory disease that causes airsacculitis and thus it does not usually result in significant condemnations at processing plants. However, ILT may result in increased deaths during transportation (DOAs) and certainly in significant mortality in the field. Overall, losses in broilers result from delayed growth, and increased mortality and feed conversion. Losses in broiler breeders and commercial layers are due primarily to drops in egg production and increased mortality. Non-vaccinal ILT is a reportable disease to the OIE and thus, outbreaks of the disease may result in costly disruptions of international trade. Outbreaks of ILT tend to be recurrent in some countries, with intervals that vary substantially between outbreaks. The use of chicken embryo origin (CEO) vaccines has often been considered a source of the outbreaks, albeit not all outbreaks are related to CEO vaccines. CEO vaccines continue to be used successfully in broiler breeders and layers in many countries, despite the concern of latency and potential reversion to virulence. Where CEO vaccines are used and applied as originally designed (individual eye drop application), they usually provide excellent protection without causing significant problems. When CEO vaccines are given by mass application methods (spray or drinking water application) they often cause vaccinal ILT, which has contributed to the poor reputation of such vaccines. Tissue culture origin (TCO) vaccines also continue to be used successfully in many countries alone or in combination with recombinant vaccines. Recombinant Fowlpox-ILT (FPV/ILT) vaccines were first introduced in the United States market on or around 2006, whereas recombinant HVT/ILT vaccines were introduced on or around September of 2007. The use of both types of recombinant vaccines in broiler chickens has

replaced almost completely the use of live attenuated vaccines (CEO) in broilers, resulting in a drastic reduction of the number of reported cases of ILT in broiler chickens. Depending on the location and industry makeup, broiler breeders and commercial layers may be vaccinated with recombinant vaccines, but most often with live attenuated vaccines (TCO or CEO) if they are legal and available. Many countries have banned the use of CEO vaccines, and some have prohibited the use of any live attenuated vaccines. Few countries use killed ILT vaccines (e.g. Peru, Ecuador, Colombia) without palpable or demonstrable evidence of the efficacy of such products. ILT is mostly under control thanks to sound vaccination programs, enhanced biosecurity practices, reduced mass application of live attenuated vaccines, coordinated industry responses and effective interventions in the face of field outbreaks. New recombinant vaccines have been introduced in various markets to include vectors such as Newcastle disease virus (NDV) and to produce recombinant vaccines with vectors that provide a level or protection against the vector itself, ILT and other disease agents such as Newcastle disease virus.

Overall, both MD and ILT continue to be represented in the form of isolated and scattered outbreaks that should be interpreted as a reminder that although both diseases are under control, both depend on a delicate balance between poultry genetics, husbandry, biosecurity and proper vaccination.

Live Merck/MSD Discussions Session 2

Session 2: ILT Vaccination

Moderated by: Dr. Maricarmen Garcia

1) DURATION OF IMMUNITY IN CHICKENS VACCINATED WITH INNOVAX-ND-LT AND INNOVAX-ND-IBD

Julio Cruz-Coy, Linda Gergen, Marielle van Hulten, Rik Koopman, and M. Morsey
MSD AH and Merck AH

Innovax-ND-LT and Innovax-ND-IBD are commercially available dual recombinant vaccines based on the Herpes Virus of Turkeys (HVT). Innovax-ND-LT contains the fusion (F) gene from Newcastle disease virus and the gD plus gI genes from Infectious Laryngotracheitis virus, whereas Innovax-ND-IBD contains the fusion (F) gene from Newcastle disease virus and the VP-2 gene from infectious bursal disease virus. The present report describes results from experiments that were conducted to demonstrate the duration of immunity induced by these vaccines against NDV and ILTV when these vaccines were used alone or in combination with serotype 1 Marek's disease vaccine (CVI988). Chickens vaccinated with a single dose of Innovax-ND-LT at 1 day of age were highly protected from challenge with the velogenic NDV (GB Texas) and ILTV (LT 96-3) strains for 60 weeks post vaccination. In addition, chickens vaccinated with a single dose of Innovax-ND-LT + CVI988 at 1 day of age were highly protected from challenge with NDV (GB Texas) at 60 weeks of age. Similarly, Chickens vaccinated with a single dose of Innovax-ND-IBD + CVI988 at 1 day of age were highly protected from challenge with NDV (GB Texas) at 60 weeks of age. These results demonstrate that Innovax-ND-LT and Innovax-ND-IBD provide long-lasting immunity against ILTV as well as NDV, and this duration of immunity is also achieved when these vaccines are used in combination with Serotype 1 Marek's vaccines.

2) DYNAMICS OF INFECTIOUS LARYNGOTRACHEITIS VIRUS TRANSMISSIBILITY AMONG FOWLPOXVIRUS-VECTORED-LARYNGOTRACHEITIS VACCINATED AND NAIVE SPF BIRDS

Fernando Lozano[1], Timea Tatar-Kis[2], and John Elattrache[1]
[1]Ceva Animal Health, USA
[2]Ceva Animal Health, Hungary

Dynamics of transmissibility of infectious laryngotracheitis virus (ILTV) challenge among Fowlpoxvirus-vectored-Laryngotracheitis (rFPV-gB/LT) vaccinated and non-vaccinated SPF birds was conducted under isolation conditions. Birds were distributed into three groups: Group 1: Wing-web vaccinated and Challenged (n=30); Group 2: Positive control (n=20); Group 3: Negative/Contact control (n=10/10). Birds vaccinated were sub-distributed into Vaccinated-Challenged (Vac-Ch) (n=20); Vaccinated-Contact (Vac-Ct) (n = 10) plus Non-vaccinated-contact birds (Ct) (n=10) and commingled in the same isolation unit. Vaccine rFPV-gB/ILT was administered at 4 weeks of age and challenged at 8 weeks of age with a USDA virulent ILTV strain. Assessment parameters included clinical signs, mortality, LT serological response, and qPCR on feather pulp. A 10-day daily observation period post-challenge was conducted. No LT clinical signs in the Vaccinated – Challenged group were observed in 85% of the birds. Vaccinated-Non-challenged birds were 100% clinically healthy. Non-vaccinated – naive contact birds commingled with the Vaccinated-Challenged birds had 50% clinical ILT. All birds in the positive control group had clinical signs of ILT. The negative control group was clinically normal. A higher serological response using specific LT gB Elisa was detected at the end of the post-challenge period in vaccinated birds. Detection of ILTV in feather pulp by qPCR was 90% positive in the positive control group. The vaccinated group had positive qPCR detection in feather pulp in 10% (Vac-Ch); 30% (Vac-Ct); and 30% (Non-Vac-Ct). Vaccination with rFPV-gB/ILT vectored vaccine protected birds against ILTV challenge.

3) ANTIBODY RESPONSE TO THE gB GLYCOPROTEIN AS AN INDICATOR OF "TAKE" AND PREDICTOR OF EFFICACY AGAINST ILT CHALLENGE, AFTER IMMUNIZATION WITH A LIVE rFP-LT VACCINE

Zsuzsanna Gyuró[1], Miklós Kopecsnik[1], Anna Gazdag[1], Albert Tóth[1], Jean-Cassien de Foucauld[2], László Makranszki[1], and Zoltán Pénzes[1]
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Vectormune® FP ILT+AE vaccine consists of a live, vector fowlpox virus (rFP-LT) expressing the membrane fusion protein (gB) and the encapsidation protein (UL-32) of avian infectious laryngotracheitis virus (ILTV), and a live avian encephalomyelitis virus (AEV).
Commercial layer and layer-type SPF pullets (20 and 10 pullets) were vaccinated with a minimum dose of Vectormune® FP ILT+AE vaccine at 8 weeks of age and challenged intratracheally with an ILTV strain at 11 and at 65 weeks of age, respectively, together with non-vaccinated controls.
Five and seven days after vaccination, the vaccine application site was examined individually for a positive local reaction as evidence of poxvirus vaccine "take". Blood samples were taken from each bird on the challenge day for gB and gI ILTV serology measured with commercial ID Vet ELISA kits. After challenge, animals were observed individually for one week. Tracheal lesions were scored after necropsy.

100% "take" of Vectormune® FP ILT+AE vaccine was confirmed with both the evaluation of skin reaction to FPV and ILTV serological response. 83.3% (25/30) of seropositive birds for gB were protected against ILTV challenge, whereas 96% (48/50) of seronegative birds for gB were not protected. All vaccinated and control birds were gI seronegative until challenge.
The gB ILTV humoral antibody titre was shown to be a reliable indicator of vaccine "take", and a promising predictor of protection against ILTV challenge conferred by vaccination with this rFP-LT+AE (gB) vaccine. Together with the gI serology, it provides a tool for differentiation of infected from vaccinated animals (DIVA).

4) RECONSTRUCTION AND MUTAGENESIS OF AVIAN INFECTIOUS LARYNGOTRACHEITIS VIRUS FROM COSMID AND YEAST CENTROMERIC PLASMID CLONES

Stephen Spatz[1], Maricarmen García[2], Walter Fuchs[3], and Taejoong Kim[1]
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The genomes of numerous herpesviruses have been cloned as infectious bacterial artificial chromosomes (BAC); however, attempts to clone the full genome of infectious laryngotracheitis virus (ILTV) have met with limited success. This lack of an infectious clone has impeded the understanding of gene function. In this study, we report the development of a cosmid/yeast centromeric plasmid (YCp) based genetic system to reconstitute ILTV. Overlapping cosmid clones were generated that encompassed 90% of the 151-Kb genome of the ILTV strain USDA. Viable ILTV was produced by cotransfection of leghorn male hepatoma (LMH) cells with these cosmids and a YCp recombinant containing the missing genomic sequences - spanning the TR_S/U_L junction. An expression cassette for green fluorescent protein (GFP) was inserted within the redundant inverted packaging site (ipac2), and the cosmid/YCp-based system was used to generate recombinant replication-competent ILTV. Viable virus was also reconstituted with a YCp clone containing a *BamH*I linker within the deleted ipac2 site, further demonstrating the nonessential nature of this site. The three reconstituted viruses replicated in chicken kidney cells with growth kinetics and to titers similar to those of the parental wildtype ILTV-USDA strain. Specific pathogen-free chickens inoculated with the reconstituted ILTV succumbed to clinical disease to similar levels as birds inoculated with wildtype virus, demonstrating the reconstituted viruses retained their virulent phenotypes. This cosmid/YCp-based genetic system will be useful to investigate the effect of viral mutations on ILTV pathogenesis and latency and thus advance the development of improved vaccines.

5) ASSESSMENT OF ACTIVATED AND REGULATORY T CELLS IN THE TRACHEA OF NON-VACCINATED AND VACCINATED CHICKENS AFTER EXPOSURE TO INFECTIOUS LARYNGOTRACHEITIS VIRUS (ILTV) CHALLENGE

Daniel Maekawa[1], Patrick Whang[1], Sylva Riblet[1], David Hurley[2] and Maricarmen García[1]
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Even though, protection efficacy among the ILTV vaccines is well established, little is known about the components of the immune responses associated with resistance and modulation of the disease. Early ILTV studies have pointed out the importance of the T cell mediated immune response for the control of ILTV. However, assessment of activated and regulatory T cells at primary sites of viral replication has not been conducted. The aim of this study was to evaluate the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells and quantify the presence of regulatory T cells in the larynx-trachea of chickens vaccinated with chicken embryo origin (CEO), tissue culture origin (TCO) and recombinant Herpesvirus of Turkey-Laryngotracheitis (rHVT-LT) after challenge with ILTV. Results indicated that CEO vaccine conferred complete protection based on the ability of CEO vaccinated chickens to block challenge virus replication, prevent trachea lesions and clinical signs. The T cell response in the trachea of CEO vaccinated chickens was characterized by an early increase and activation of CTLs. A significant correlation was found between increase of activated CTLs and decrease of clinical signs. The TCO and rHVT-LT vaccines were categorized to induce partial protection by their capacity to reduce but not block the replication of the challenge virus, trachea lesions and clinical signs, while a moderate increase of resting and activated CTLs and NK cells appeared in the larynx-trachea. Regulatory T and NK cells were significantly increased in the non-vaccinated challenged group

6) EFFICACY OF TURKEY HERPES VIRUS RECOMBINANT VACCINE (rHVT-LT) AGAINST A GENOTYPE VI CANADIAN INFECTIOUS LARYNGOTRACHEITIS VIRUS

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[5]Provincial Laboratory for Public Health, Calgary, Alberta, Canada
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Infectious laryngotracheitis virus (ILTV) is endemic in backyard chicken in Alberta. Recent studies have shown that ILTV wild-type strains are the second most common cause of infectious laryngotracheitis (ILT) outbreaks in Alberta's backyard flocks. However, poultry flocks in Alberta are vaccinated against ILTV infection sparsely; only commercial parental flocks and 13% of backyard flocks are vaccinated. The Ministry of Agriculture and Forestry recommends the use of tissue culture origin vaccines and recombinant viral vector vaccines in the face of ILT outbreaks. The objective of this study was to determine the efficacy of a turkey herpes virus recombinant vaccine (rHVT-LT) against a Canadian wild-type genotype VI ILTV strain. At 1 day of age, 44 chickens were separated into four equal groups. Two of those groups were vaccinated while the remaining groups were mock vaccinated. At 3 weeks of age, one of the vaccinated groups and one of the mock vaccinated groups were challenged with ILTV. For 2 weeks, the chickens were observed twice a day for clinical signs. At 3, 7, 10 and 14 days post-infection (dpi), bodyweight, feather tips, cloacal and oropharyngeal swabs were collected. At 5 and 12 dpi, blood was collected to quantify CD8+ and CD4+ T cells. At 14 dpi, the chickens were euthanized, and tissue samples were collected. Results showed that the vaccine prevented weight loss, reduced viral shedding, and increased CD8+ T cells in early infection in the vaccinated chickens without reducing clinical signs. Overall, the vaccine protected the chickens against ILTV infection partially.

Session 2: MDV Vaccination

Moderated by: Dr. Stephen Spatz

1) NEW GENERATION MAREK'S VACCINE, RN1250 STRAIN SEROTYPE ONE-ORIGINATED, AS A BASE OF HATCHERY VACCINATION PROGRAMS

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Marek's disease (MD) has been recorded for decades as one of the major diseases of poultry. This Gallid Alphaherpesvirus 2 that targets various lymphocytes subpopulations of the chicken induces tumor formation and immunosuppression. Vaccination has been the most relevant tool together with cleaning & disinfection and/or biosecurity measures to help reduce the incidence of the disease worldwide. Vaccination programs against Marek's disease have evolved over the years to improve control of field strains of increased virulence worldwide. The HVT vaccine was the very original one to be used, followed by the serotype 2 SB-1 and the serotype 1 Rispens CVI988 strains, used singly or in combination (for the CVI988) with HVT and HVT vectors. A new generation of Marek's vaccine was licensed in the USA in 2016. The vaccine strain is a hybrid virus that combines CVI988 with the RM1 and Md5 strains of the Marek's disease virus. The vaccine has shown protective ability in various segments of poultry production, broilers, broiler breeders and layer pullets in combination with HVT vectored vaccines of Newcastle disease and of infectious bursal disease. Results of field monitoring of combinations of vaccines take as well as results of controlled experimental conditions-type of clinical studies justify the introduction of a new generation of serotype 1 vaccine to the poultry industry.

2) NEWCASTLE DISEASE VIRUS (NDV) RECOMBINANT EXPRESSING A MAREK'S DISEASE VIRUS (MDV) IMMUNOGEN PROTECTS CHICKENS AGAINST MDV AND NDV CHALLENGES

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Marek's disease (MD) is a highly contagious viral neoplastic disease of chickens caused by *Gallid alphaherpesvirus* 2, resulting in significant economic losses to the poultry industry worldwide. The commonly used *Meleagrid alphaherpesvirus* 1-based vectored vaccines are expensive to produce and difficult to handle due to the requirement of liquid nitrogen for manufacturing and delivering frozen infected cells that are viable. This study aimed to develop a multivalent MD vaccine that can be lyophilized, stored, and transported at amble temperature. To this end, a Newcastle disease virus (NDV) LaSota vaccine strain-based recombinant virus expressing MDV glycoprotein B (rLS/MDV-gB) was generated using reverse genetics technology. The biological assessments showed that the rLS/MDV-gB virus was slightly attenuated *in vivo* yet retained similar growth kinetics and virus titers *in vitro* compared to the parental LaSota virus. Vaccination of leghorn chickens (Line $15I_5x7_1$) with this recombinant virus via intranasal and intraocular routes conferred significant protection against virulent MDV challenge and complete protection against velogenic NDV challenge. These results demonstrated that the rLS/MDV-gB virus is a safe and efficacious bivalent vaccine candidate that can be potentially administered en masse via aerosol or drinking water to large chicken populations at a low cost.

3) RAPID GENERATION OF MULTIVALENT HERPESVIRUS VECTORED VACCINES USING CRISPR/CAS9 SYSTEM

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Herpesvirus of turkeys (HVT) has been shown to be an effective viral vector for generation of recombinant vaccines that deliver immunogenic genes of avian pathogens such as infectious bursal disease virus (IBDV), avian influenza virus (AIV), Newcastle disease virus (NDV) and infectious laryngotracheitis virus (ILTV). Presently, most of the commercial HVT vectored vaccines carry single foreign gene that induce dual protection against the corresponding pathogen along with Marek's disease (MD), against which HVT is used as an effective vaccine. To avoid the interference between individual recombinant HVT vaccines expressing different foreign genes, multivalent HVT recombinants capable of inducing simultaneous protection against multiple avian pathogens would be a better option. Previously, we have developed a rapid and efficient CRISPR/Cas9-mediated genome editing pipeline for generating recombinant HVT. Here we extend the application of this efficient gene editing system to generate multiple gene insertion HVT vectored virus. The insertion, protein expression of the inserted genes and the stability of each insert was evaluated by PCR, immunostaining and western blot. Successful generation of this multiple insertion HVT recombinant vaccine demonstrate the potential of CRISPR/Cas9 gene editing pipeline for rapid generation of multivalent viral vector vaccines.

4) DEVELOPMENT OF A NEW TRIVALENT HVT-VECTORED VACCINE AGAINST MAREK'S DISEASE, INFECTIOUS BURSAL DISEASE AND INFECTIOUS LARYNGOTRACHEITIS

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Infectious Laryngotracheitis (ILT), Infectious bursal disease (IBD) and Marek's disease (MD) are important diseases in commercial poultry, currently controlled with vaccination. The use of HVT-vectored vaccines has proven to be an effective tool for several diseases of poultry, with a well-known safety profile, hatchery application and long-term protection. However, due to interference issues, it is not possible to administer two HVT vaccines together, creating problems when designing a vaccination program. Aiming to provide a solution to control MD, ILT and IBD with a single vaccine, a new and unique recombinant vHVT317-IBD-ILT construct has been developed. This dual-insert construct has a vHVT013 (VAXXITEK HVT+IBD) backbone with one additional insert expressing a glycoprotein D (gD) gene from ILTV. Both IBDV and ILTV genes are expressed from a single promoter. The vHVT317-IBD-ILT construct was shown to be safe for chickens, non-target avian species and mammalian species. The construct was also genetically stable after five *in vitro* passages, and it did not revert to virulence after five back passages in chickens. The construct had similar tissue tropism and environmental safety as the HVT parental virus. Efficacy was demonstrated after either subcutaneous or *in ovo* vaccination, against virulent MD, IBD and ILT challenges. The unique vHVT317-IBD-ILT vector vaccine provides an additional tool for poultry veterinarians, allowing for control of MD, IBD and ILT with a single vaccination, and providing flexibility when designing the optimal vaccination program adapted to their specific epidemiological situation.

5) CONSTRUCTION, SAFETY, STABILITY, AND EFFICACY OF A RECOMBINANT HVT-ND VACCINE

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A recombinant HVT-ND (Herpes Turkey Virus-Newcastle Disease) was developed as a bi-valent vaccine for protection against Marek's disease (MD), a common cause of condemnations and immune suppression in broilers and Newcastle disease (ND), a highly contagious and fatal disease affecting numerous species of birds including poultry. The HVT-ND recombinant vaccine was developed and tested for target gene expression, safety, stability and efficacy. Safety was established after administration of 10X the vaccine dose via the *in ovo* route to 18-day-old embryos (E18) and the subcutaneous route to day-of-age (D0) chickens followed by 120 days of observations. No adverse effects on hatchability and body weight and no clinical signs or lesions consistent with MD were observed in vaccinated chickens. Vaccine stability was confirmed by PCR and immunofluorescence of the targeted gene after five consecutive passages *in-vivo* and *in-vitro*. Efficacy of recombinant HVT-ND against vMDV (GA22) was demonstrated in SPF leghorns chickens for both the *in ovo* route at E18 (83%) and the subcutaneous route at D0 (80%). Full protection against velogenic NDV challenge was also demonstrated in SPF and broilers birds.

6) THE EFFECTS OF ADMINISTRATION OF PROBIOTICS WITH MAREK'S DISEASE VACCINE AGAISNT CHALLENGE WITH VERY VIRULENT MAREK'S DISEASE VIRUS IN CHICKENS

Jegarubee Bavananthasivam[1,2], Jake Astill[1], Ayumi Matsuyama[1], Mohammadali Alizadeh[1], Nadiyah Alqazlan[1], Bahram Shojadoost[1], and Shayan Sharif[1*]
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The first commercial vaccine developed against a neoplastic disease was Marek's disease (MD) vaccine. Since then several MD vaccines have been used to control MD in chickens. However, the emergence of new strains of Marek's disease virus (MDV) imposes a threat to vaccine protection. Therefore, the current study was carried out to investigate whether administration of probiotics enhances the efficacy of Herpesvirus of turkeys (HVT) vaccine to induce protective immunity against MDV infection. In this regard, a probiotic cocktail comprised of four Lactobacillus species was administered with HVT at embryonic day 18 (ED18) and/or from day 1 to day 4 post-hatch. The results indicated that the administration of probiotics with HVT at ED18 and oral gavage of probiotics from day 1 to day 4 increased the expression of major histocompatibility complex (MHC)-ll on macrophages and B cells in spleen. Interestingly, the number of CD4+CD25+ T regulatory cells was also reduced in spleen. Subsequent infection with MDV induced increased expression of interferon (IFN)-β gene in cecal tonsils at 4 days post-infection (dpi) and INF-α gene in spleen at 21dpi. Moreover, the expression of tumor growth factor (TGF)-β gene was also reduced in the group that received both vaccine and probiotics. Although the administration of probiotics contributed to enhance the host responses, it only moderately improved the efficacy of HVT and reduced tumor incidence from 35.7% to 16.7% in MDV infected chickens. The results of this study provide evidence that probiotics may have an impact on immunity conferred by MD vaccines.

7) GUT MICROBIOME IS ASSOCIATED WITH IMMUNITY AGAINST MAREK'S DISEASE VIRUS INFECTION IN CHICKENS

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Marek's Disease Virus (MDV) infects chickens via respiratory route and causes lymphomas in internal organs including gastrointestinal tract. Recently, there has been an interest in understanding the connection between the gut microbiome and immunity to infectious diseases. MDV infection causes a shift in the gut microbiome composition. However, the interaction between the gut microbiome and immune responses against MDV infection is not well understood. Therefore, the current study was carried out to understand the impact of the gut on MDV pathogenesis. In this regard, chickens were administered with a cocktail of antibiotics to deplete the gut microbiome and were subsequently infected with MDV. The findings showed that interruption in the gut microbiome increased the severity of the disease in experimentally challenged chickens. In addition, an increase of interferon (IFN)-α, IFN-β and IFN-γ transcription in the bursa of Fabricius at 4 days post-infection was observed. This suggests that these genes might have been involved in the host response against MDV infection. The observations in this study shed more light on the association between the gut microbiome and MDV infection in chickens. More research is needed to explore the underlying mechanisms of involvement of the gut microbiome in immunity against Marek's disease.

AS/AU Live Session 2: Related Field Works. Sponsored by: Merck/MSD

Moderated by: Dr. Joanne Devlin

1) MONITORING OF AVIAN HERPESVIRUSES IN DUST SAMPLES – PRACTICAL ASPECTS

Priscilla F. Gerber[1], Trong Van Nguyen[2], Md. Ahaduzzaman[1], Awol M. Assen[1], Peter J. Groves[3], and Stephen W. Walkden-Brown[1]

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Poultry dust samples have been used as a marker for monitoring incursion of wild-type virus and the administration of live vaccines against Marek's disease virus (MDV) and infectious laryngotracheitis virus (ILTV), but there are no guidelines for collection of this sample type. We examined the effects of 1. dust collection methods, i.e., scraped from barn surfaces or deposited in settle plates; 2. location of dust collection within poultry barns; and 3. testing of a individual dust samples or a pool of 2-6 samples from a barn on the detection of ILTV or MDV DNA viral load. Dust collection method significantly influenced the level of viral genome load detected with dust collected in settle plates reflecting the current level of viral genome in the dust while scraped samples reflect historical accumulation. There was no systematic bias in detection of MDV or ILTV genome associated with sample collection location in the barn. Testing of a single pooled sample can therefore be used for detecting MDV and ILTV when virus load is high but it may increase the chance of false negatives when virus load is expected to be low. Vaccine MDV and ILTV DNA is readily detected from dust samples 7 days after vaccine administration, and MDV and ILTV wild-type incursion can be detected in dust from unvaccinated flocks in the absence of disease. Measurement of ILTV and MDV DNA in settle plate dust provides a practical means of monitoring vaccination and wild-type virus in commercial chicken flocks.

2) VARIATIONS IN DRINKING WATER ILT VACCINATION TECHNIQUES FOR BROILER CHICKENS

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Administration of Infectious Laryngotracheitis (ILT) vaccines via drinking water to young broilers for mass vaccination is difficult and often results in in adequate protection or excessive vaccine reactions if the procedure is not optimal. Vaccination technique to achieve as many chicks successfully acquiring an immunising dose on the day of vaccination is critical.
Forty houses over 18 farms in three Australian regions were observed in their ILT vaccine administration procedures. Flock sizes varied between 14,400 and 49,560 birds. The timing of various steps in administration were recorded.

Twenty-three houses were tunnel ventilated, 15 were open-sided and 2 were open-sided free range. Twenty-eight farms were vaccinated against ILT using Serva strain and 12 used A20 strain. Vaccination age varied between 7 and 15 days. There was a variety of materials used to protect the vaccine in water with 18 houses a proprietary stabilizer containing blue dye, 14 using skim milk and 8 using combined skim milk and stabilizer. Volume of water used for vaccination varied between 11.26 and 47.9 mL/bird. Time of restriction of water prior to vaccinating varied between 3 and 134m and birds were recorded as varying from "somewhat" to "very" thirsty when water was returned. Water stabilization time prior to adding vaccine varied from 0 to 118m and time until the farmer walked the shed to activate the birds varied between 0 and 127m after vaccination began. Time to consume the vaccine varied from 36 to 226m. Association of these variables with vaccine efficiency needs to be evaluated.

3) TRANSMISSION STUDIES WITH VIRULENT AND VACCINE STRAINS OF INFECTIOUS LARYNGOTRACHEITIS VIRUS

Addisu Awukew Yegoraw[1], Shahid Nazir[1], Priscilla F. Gerber[1], and Stephen W. Walkden-Brown[1]
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Infectious laryngotracheitis virus (ILTV) is thought to exit the host in respiratory aerosols and enter by inhalation of these. High levels of ILTV DNA have been detected in excreta but it is not known whether this represents infective virus or not. We investigated the transmission of ILTV from excreta or in aerosols from infected to susceptible commercial meat chickens. Transmission of two field strains of ILTV (Classes 9 and 10) and three vaccine strains (SA2, A20 and Serva) by both methods was tested. For aerosol transmission, air from isolators containing infected birds was ducted through a paired isolator containing uninfected recipient chickens. To test transmission via excreta, putatively infective material was prepared from excreta samples containing a high level of ILTV DNA within the first week after infection either by filtration or centrifugation. Chicks were infected bilaterally by eye drop. Clinical signs were monitored daily, and choanal cleft swab samples were collected at 4, 8, 15, 22 and 28 days post-infection (DPI) in the aerosol study and at 7 and 14 DPI from the excreta transmission studies. There was no transmission of ILTV from excreta suggesting that ILTV is inactivated during passage through the gut. Aerosol transmission was efficient for the field viruses, inducing clinical signs, sustained infections and greatly elevated choanal cleft ILTV DNA levels in recipient chickens. Transmission of vaccine viruses was less efficient and only resulted positive samples at single time points. Further studies are being undertaken and will be reported at the meeting.

4) FIELD APPLICATION OF MONITORING OF INFECTIOUS LARYNGOTRACHEITIS VIRUS IN CHICKENS BY PCR DETECTION OF VIRAL GENOME IN DUST SAMPLES

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Infectious laryngotracheitis (ILT) is a significant disease for the poultry industry in Australia. We describe a field case in which qPCR detection of ILTV DNA in dust to assess vaccination status of flocks was expanded to track flock ILTV status, contributing to elimination of ILT from farms of a large integrator company. In 26 meat chicken flocks that were vaccinated with the Serva or A20 ILT vaccines at the age of 7-13 days, settled dust samples were collected at 0, 4, 7, 14 and 21 days post vaccination (dpv) using dust collectors suspended from downwires. Birds were placed on new bedding material. Unexpectedly, ILTV DNA was detected in dust samples collected before vaccination (0 dpv) in 22 of the flocks. PCR-RFLP typing of positive samples showed that the detected ILTV was different from the vaccine virus in use. To assess bird infection status 15 tracheal swabs per flock at 0 and 21 dpv and dust samples at 0, 7, 14 and 21 dpv were collected from an additional 14 flocks vaccinated with A20 vaccine. Prior to vaccination ILTV was detected in 71% (10/14) of flocks in dust samples and 46% (97/211) of the swabs. Again, typing of samples revealed presence of non-vaccine ILTV. Using these and other findings a large integrator in South Australia conducted an aggressive control program during 2019-2020 culminating in cessation of ILT vaccination in mid-2020. Subsequent dust testing of 50 flocks confirmed absence of ILTV and the integrator has remained free of ILT since.

5) PROTECTION PROVIDED BY VARIABLE VACCINATION COVER AGAINST INFECTIOUS LARYNGOTRACHEITIS CHALLENGE IN MEAT CHICKENS

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Mass vaccination of broilers with live infectious laryngotracheitis virus (ILTV) via drinking water can result in variable initial vaccine take. This study was conducted to determine the effect of partial ILT vaccination on the level of protection against virulent ILTV challenge. The experiment had a 4x3 factorial arrangement testing 4 levels of ILTV vaccination (0%, 7%, 20% or 100% of chicks eye drop inoculated with A20 vaccine) and 3 levels of virulent ILTV challenge (no challenge or challenge at 7 and 25 days post vaccination, dpv). Day old Cobb broiler chicks were placed in 20 isolators (15/isolator) with the ILTV challenge treatments replicated in two isolators and the sham challenge treatments not replicated. ILTV challenge was by introduction of two chicks per isolator eye drop inoculated with virulent ILTV or normal saline 4 days earlier. Mortality and clinical signs were recorded daily and birds were weighed weekly to 46 days of age. Choanal cleft swabs were collected at 4 and 7 dpv and weekly thereafter for qPCR detection of ILTV. Protection against ILT associated mortality/euthanasia was 47%, 85% and 82% for 7 dpv and 64%, 85% and 50% for 25 dpv challenge groups in 7%, 20% and 100% vaccinated birds respectively. Mean total clinical scores were 11.1, 10.6, 7.3 and 0.9 for 7 dpv and 29.6, 14.9, 5.8 and 5.1 for 25 dpv challenge groups in 0%, 7%, 20% and 100% vaccinated birds respectively. Partial vaccination provided unexpectedly high levels of protection. Additional data will be presented at the symposium.

6) VAXSAFE ILT PROVIDES LONG TERM PROTECTION AGAINST CHALLENGE WITH AN ENDEMIC AUSTRALIAN VIRULENT STRAIN

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Infectious Laryngotracheitis (ILT) virus is a highly contagious respiratory virus of chickens and pheasants which can induce significant morbidity and mortality. While biosecurity plays an important role in limiting ILT infection, vaccination remains the cornerstone to preventing significant flock losses. Live vaccines have been shown to provide the best protection against ILT and, as part of the fundamentals of vaccine registration, we explored the duration of immunity provided by a vaccine candidate we have developed which is deficient in glycoprotein G (Vaxsafe ILT). Here we demonstrate that eye-drop vaccination of 7-day old chickens with Vaxsafe ILT, induced efficacious protection for at least 20 weeks, with significant reductions in the severity of clinical signs, tracheal pathology and mortality. These data provide a foundation for further elucidating the duration of immunity provided by Vaxsafe ILT and underscore the use of Vaxsafe ILT as an effective means to prevent outbreaks in commercial flocks.

7) LACK OF IMMUNOHISTOCHEMICAL STAINING IN SKIN AND FEATHER FOLLICLES OF CHICKENS INFECTED WITH INFECTIOUS LARYNGOTRACHEITIS

Shahid Nazir[1], Richard P.G. Charlesworth[2], Stephen W. Walkden-Brown[1], and Priscilla F. Gerber[1]
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Feathers have been used to detect nucleic acids of several viruses of chickens including infectious laryngotracheitis virus (ILTV) and Marek's disease virus (MDV), and to detect MDV antigens by immunohistochemistry (IHC). Although the association of MDV with chicken skin and feathers is well-established, this has not been investigated for ILTV. In this study we examined sections of skin containing feather follicles and pulp of 30 chickens inoculated with an ILTV field strain and 5 sham-inoculated chickens stained for ILTV by IHC. The frequency of feather follicles with high melanin was relatively low in white feathered chickens (6%, 1/15 White Ross broilers) and moderately high in brown feathered chickens (40%, 6/15 ISA Brown layers). This can be erroneously interpreted as evidence of antigen detection due to a reaction of melanin with the DAB chromogen used for IHC staining. Therefore, we developed a method for melanin-bleaching using potassium permanganate followed by incubation with oxalic acid prior to immunostaining. This technique did not affect the antigenicity of ILTV antigen as confirmed by the lack of difference IHC positive signals in bleached and non-bleached conjunctiva tissues known to contain ILTV antigen and that have no endogenous melanin. To our knowledge, this is the first report of a melanin-bleaching technique prior to IHC of chicken feathers for detection of pathogens. None of the sections of skin and feather follicle of ILTV-infected birds were positive for ILTV antigen by IHC. Further studies are necessary to determine the source of ILTV DNA detected in feather by PCR.

Pre-recorded Keynote Speaker: Dr. Helen Sang. Sponsored by: Ceva Animal Health Inc.

The Roslin Institute, University of Edinburgh

Moderated by: Alice Wang

Dr. Helen Sang is a Principal Investigator at the Roslin Institute of the University of Edinburgh. She was appointed Personal Chair in Vertebrate Molecular Development in 2009. She is a Fellow of the Royal Society of Edinburgh and Fellow of the Royal Society of Biology. Her current research interests include aapplications of transgenesis in the chick, particularly in study of the development of the chick embryo and resistance to disease.

APPLYING GENOME ENGINEERING IN THE CHICKEN TO THE UNDERSTANDING OF HOST/PATHOGEN INTERACTIONS

H.M. Sang
The Roslin Institute, University of Edinburgh, UK

Genome engineering encompasses the several technologies that may be used in the chicken to add transgenes, delete or knockout genes or make subtle genetic changes. Transgenes have been added using lentiviral and transposon vectors and we have developed reporter lines in which, for example, cells of the macrophage lineage express fluorescent proteins. These reporter lines can be used in many ways to explore specific immune cell lineages *in vivo*, including investigating interactions with pathogens. In the past few years a very efficient technology has been developed based on the *in vitro* culture of primordial germ cells (PGCs), the precursors of the male and female gametes, that enables specific gene edits to be made in targeted genes. PGCs are modified in culture and then injected into sterile host embryos that produce sperm and eggs from the edited PGCs (M. McGrew). These advances facilitate production of gene edited birds within one year. These tools may be utilised to investigate host/pathogen interactions as exemplified by investigation of the function of ANP32A in replication of avian influenza (AI). Long, Barclay and colleagues identified ANP32A in a study to investigate cellular proteins that interact with AI proteins and found that chicken ANP32A has an inserted 33 amino acid sequence that is required for AI replication. We utilised CRISPR-Cas9 gene editing to delete this sequence in cells and showed that they not support AI replication (Long, Idoko-Akoh and colleagues). The technology to generate birds from edited PGCs has the potential to generate chickens resistant to major pathogens.

Live Ceva Discussion Session 3

Session 3: Molecular Virology Panel 1

Moderated by: Dr. Jiuzhou (John) Song

1) MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF EXTRACELLULAR VESICLES RELEASED BY A LYMPHOCYTE LINE TRANSFORMED BY MAREK'S DISEASE VIRUS

Laëtitia Trapp-Fragnet[1], Sylvie Rémy[1], Valérie Labas[2], Lucie Combes[2], Ana-Paula Texeira[2], Julien Gaillard[3,] and Caroline Denesvre[1]

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Extracellular vesicles (EVs) emitted by cells allow cell-to-cell transmission of bioactive molecules (proteins, DNA, mRNA and/or microRNAs) leading to an exchange of informations between cells and a potential reprogramming of “recipient cells”. Tumor cells are able to release a significant amount of EVs that can influence tumor development, tumor growth, metastatic processes and resistance to cancer therapies. EVs produced by tumor cells are able to transmit signals to immune cells and thus contribute to the escape of cancer cells to the immune response and to the establishment of an immunosuppressive microenvironment favorable to tumor growth. Despite the identification of pro-oncogenic viral molecules, the processes leading to MDV-induced tumorigenesis remain poorly understood. We assessed the role of EVs produced by MDV-transformed lymphocytes in neoplastic mechanisms by specifying their impact on cell proliferation. Molecular characterization of EVs isolated from MDV tumor cells was performed by western blot and proteomic approach. We validated the presence of EV-specific markers, identified cellular biomolecules enriched in these EVs and showed that EVs do not contain viral proteins. Moreover, we demonstrated that EVs are rapidly internalized by recipient cells and that they are able to modulate avian immune cells proliferation. These EVs have indeed a pro-proliferative effect on splenic T cells and an antiproliferative effect on B cells isolated from bursa of Fabricius. These data suggest that EVs are able to transfer opposite signals to uninfected B and T cells, affecting their proliferation and therefore that they could contribute to transformation process.

2) THE ROLE OF THE REPEAT REGIONS IN MAREK'S DISEASE VIRUS REPLICATION AND PATHOGENESIS

Tereza Vychodil, Andelé Condradie, Luca Bertzbach, and Benedikt Kaufer
Freie Universität Berlin

The Marek's disease virus (MDV) genome consist of two unique regions that are flanked by inverted repeat regions. These repeat regions harbor a number of genes that play an important role for MDV replication and pathogenesis. Until now, it remains elusive why MDV and other herpesviruses harbor these duplicated regions in their genome. It has been hypothesized that either duplication of the genes encoded in the repeats or repeat-mediated inversions of the unique regions are important for these herpesviruses. In the present study, we set to determine the role of gene diploidity and isomerization in MDV replication and pathogenesis. We could demonstrate that MDV mutants lacking one entire repeat region (ΔIRLS) efficiently replicate and spread from cell-to-cell in vitro. In contrast, in infected chickens, ΔIRLS replication was impaired and only few animals infected with ΔIRLS developed disease and tumors when compared to the respective controls. Beyond that, we generated recombinant viruses that harbor deletions of most of one repeat region (ΔIRLS-HR), only leaving short terminal sequences of the repeats behind. These remaining repeats sequences facilitated a rapid restauration of the respective repeat by homologous recombination, thereby serving as excellent platform viruses that can be used for a simple manipulation of genes encoded in the repeats. Taken together, our study provides the first evidence that MDV requires both copies of the repeats for efficient replication and pathogenesis in chickens. Furthermore, we established platform viruses that can be used for a rapid manipulation of any diploid MDV gene.

3) TURKEY HERPESVIRUS VECTORED VACCINE EXPRESSING THE HEMAGGLUTININ GENE FROM THE HPAI VIRUS A/CHICKEN/GUANAJUATO/07437-15/2015(H7N3) SHOWS PROTECTION AGAINST HOMOLGOUS CHALLENGE

O.B. Faulkner, L. Frizzo da Silva, B. Kobilarcsik, S. Sheen, M. Esaki, D. Perez, D. Rajao, Z. Penzes, and K. Moore Dorsey
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Highly pathogenic avian influenza (HPAI) caused by the subtype H7N3 is currently a severe issue in Mexico. Avian influenza viruses constantly mutate due to antigenic shift and drift with conventional vaccine regimens. A vectored vaccine was developed for Mexico using the herpesvirus of turkeys (HVT) expressing the *hemagglutinin* (HA) gene from a HPAI virus A/chicken/Guanajuato/07437-15/2015 (H7N3) isolated from an outbreak in Mexico in 2015, referred to here as rHVT/AI H7. Stability of the HA gene was confirmed by sequence analysis of the gene insert and its flanking regions after seven passages in chicken embryo fibroblast (CEF) cells. Western blot and immunofluorescence assays were demonstrated for HA gene stability after seven passages in CEF at protein expression level. Efficacy was determined by vaccinating day of age chickens with rHVT/AI H7 by subcutaneous injection. No morbidity, mortality, or adverse reactions due to the vaccine were observed. Chickens were challenged at five weeks of age with 10^6 EID_{50} A/chicken/Mexico/CIP-102_RGSCG04/2016 H7N3 Mexico virus strain. Oral and cloacal swabs were collected 2- and 4-days post challenge to assess viral shedding. The rHVT/AI H7 showed 100% protection against the homologous H7N3 HPAI challenge. The positive control chickens all developed clinical signs associated with H7N3 resulting in 100% mortality. This rHVT/AI H7 vaccine showed efficacy against the homologous challenge, and was licensed by the Mexican Government (SAGARPA) in 2017.

4) TELOMERIC REPEATS ARE CRITICAL FOR HVT INTEGRATION AND FAVOR HVT PERSISTENCE AT HIGH LEVEL INTO HOST

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The herpesvirus of turkey (HVT) is commonly used as a vaccine vector to protect chickens against important avian pathogens. HVT establishes latency into host and harbors telomeric repeat arrays (TMRs) at the end of its genome, like Marek's disease virus (MDV). We have previously shown that MDV TMRs facilitate integration of the virus genome into host telomeres, a state that also allows efficient mobilization of the viral DNA during reactivation. Therefore, we investigated if HVT also integrates efficiently into host telomeres and if the TMR play a role in HVT persistence *in vitro* and *in vivo*. We generated a HVT mutant that lacks both TMRs (HVTΔTMR). This mutant efficiently replicates in cell culture, but that its integration was severely impaired in lymphocytes *in vitro*. To assess the role of the TMRs in replication and latency *in vivo*, one-day-old chicks were infected with HVTΔTMR or WT HVT. HVT ΔTMR loads were significantly reduced in PBMCs and much more strongly in feather materials. Finally, HVT loads were greatly reduced in the spleen of HVTΔTMR at the end of the experiment as well as reactivation from splenocytes, suggesting that the establishment of latency and reactivation was also impaired compared to WT. Taken together, these findings indicate that the TMRs promote the persistence of the viral genomes in PBMCs and spleen, contribute to the transport of the virus to the feather follicles and potentially also virus reactivation.

5) EFFECT OF SERUM EXOSOMES FROM VACCINATED AND PROTECTED AND TUMOR-BEARING CHICKENS ON IMMUNE FUNCTION

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Exosomes are small (30 – 100 nm), enveloped vesicles produced by cells and present in high concentrations (~0.5 – 5 x10^{10}/ml) in all biological fluids. We have previously identified miRNAs, mRNAs and proteins within the serum exosomes of chickens vaccinated and protected from Marek's disease virus (MDV) challenge (VEX), and from tumor-bearing chickens (TEX). Comparative analysis of these samples predicted that VEX contained miRNAs that targeted cellular proliferation pathways, as well as mRNAs mapping to the MDV genome. These data suggested that a mechanism of systemic protection against MDV-mediated tumor formation may be through exosomal targeting of cellular proliferation pathways and through the systemic presentation of MDV proteins via uptake and translation of exosome-borne mRNAs. Additionally, TEX-borne miRNAs were predicted to target phosphatidylinositol signaling, suggesting that one mechanism of MDV-mediated immune suppression may be through the targeting of T-cell activation. To test these hypotheses, we have purified exosomes via size-exclusion chromatography in sterile PBS and characterized these via transmission electron microscopy (TEM), and nanoparticle tracking analysis (NTA). We are currently examining the uptake of VEX and TEX, and the effects of this uptake on changes in total proteomes, surface MHC-I-mediated peptide presentation, and the capacity of antigen presenting cells to elicit MDV-specific T-cell activation after exosome uptake. We are also examining the effects of both VEX and TEX on cellular responses to innate agonists via kinome analysis. Finally, we are examining the capacity of VEX and TEX to serve as vaccine adjuvants, and their capacity to block vaccine-induced responses, respectively.

6) TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS OF EXOSOMES RELEASED BY MAREK'S DISEASE VIRUS TRANSFORMED T-CELL LINES

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Marek's Disease (MD) is caused by highly oncogenic alphaherpesvirus, Marek's disease Virus (MDV) which transforms primarily CD4+ T-lymphocytes. Exosomes are small (30 – 100 nm) enveloped vesicles specifically loaded and released by cells into all biological fluids. In this study, we have examined the RNA and protein content of exosomes released by T-cell lines transformed different pathotype MDVs, as well as specific recombinant MDVs. Through bioinformatic analysis of these data, we are examining the mRNAs contained, the miRNAs and their predicted targeted pathways with which they interfere, as well as their cellular and viral protein components. The underlying hypothesis of this work is that the exosome content of different pathotype MDV-transformed cells directly contributes to the virulence level of the virus *in vivo*, in terms of systemic immune suppression. To address this hypothesis, we have examined the RNA and protein contents of cell line-derived exosomes by deep-sequencing and mass spectrometry, respectively. The cell lines used were CU12 and CU47 (JM10-transformed, a vMDV), UD35 (RB-1B-transformed, a vvMDV), UD31 (N strain-transformed, a vv+MDV) and UA53 (TK strain-transformed, a vv+MDV). In addition, to address the contribution of oncoprotein Meq on exosome content, we examined the exosomes of cell lines UD36 (rMd5 strain with the RB-1B Meq isoform), UD39 (RB-1B having the CV1988-long Meq isoform) and UD40 (RB-1B having the N strain Meq isoform). With this analysis, we hope to identify not only pathotype-specific packaging of tumor exosomes, but also the contribution of Meq isoform to this differential packaging.

Session 3: Molecular Virology Panel 2

Moderated by: Dr. Sanjay Reddy

1) TARGETED GENE EDITING IN MAREK'S DISEASE VIRUS-TRANSFORMED CELL LINES USING CRISPR/CAS9 SYSTEM

Yaoyao Zhang[1], Na Tang[1,2], Jun Luo[3], Man Teng[3], Vishwanatha R.A.P. Reddy[1], Yashar Sadigh[1], Katy Moffat[1], Zhiqiang Shen[2], Venugopal Nair[1] and Yongxiu Yao[1]

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Marek's disease virus (MDV), a lymphotropic α-herpesvirus associated with T-cell lymphomas in chickens, is an excellent model for herpesvirus biology and virus-induced oncogenesis. In the lymphomas and lymphoblastoid cell lines (LCLs) derived from them, MDV establishes latent infection with limited gene expression. Although LCLs are valuable for interrogating viral and host gene functions, molecular determinants associated with the maintenance of MDV latency and lytic switch remain largely unknown, mainly due to the lack of tools for in situ manipulation of the genomes in these cell lines. Recent advances in CRISPR/Cas9-based gene editing have given opportunities for precise editing of the viral genome for identifying pathogenic determinants and studying host-virus interactions in LCLs. Here we describe the application of CRISPR/Cas9 editing approach for precise editing of the viral gene phosphoprotein 38 (*pp38*), a biomarker for latent/lytic switch, and MDV-encoded miRNA-155 ortholog miR-M4 in LCLs. Contradictory to the previous reports suggesting that pp38 is involved in the maintenance of transformation of LCL MSB-1 cells, we show that *pp38*-deleted cells proliferated at a significant higher rate, suggesting that *pp38* is dispensable for the transformed state of these cell lines. Continued proliferation of miR-M4 deleted cell lines demonstrated that MDV-miR-M4 expression is not essential for maintaining the transformed phenotype, despite its initial critical role in the induction of lymphomas. Application of CRISPR/Cas9-based gene editing of MDV-transformed cell lines in situ opens up further opportunities towards a better understanding of MDV pathogenesis and virus-host interactions.

2) NOVEL INSIGHTS IN THE ROLE OF BCL-2 HOMOLOG NR-13 (VNR-13) ENCODED BY HERPESVIRUS OF TURKEYS IN THE VIRUS REPLICATION CYCLE, MITOCHONDRIAL NETWORKS AND APOPTOSIS INHIBITION

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Bcl-2 (B cell lymphoma-2)-related gene Nr-13 plays a major role in the regulation of cell death in B-cells during the development of the avian immune system. With over 65% sequence similarity to the chicken Nr-13, herpesvirus of turkeys (HVT)-encoded HVT079 and HVT096 gene product *vNr-13* is the first Bcl-2 homolog encoded by an α-herpesvirus. HVT *vNr-13* is structurally well-conserved among the members of the Bcl-2 family proteins. Although, functional role of *vNr-13* in the context of HVT replication and interaction with the host cell has not been examined, demonstration that HVT-infected cells were relatively more resistant to serum starvation, suggested that expression of *vNr-13* could be involved in protecting the cells. Here we describe CRISPR/Cas9-based editing of exon 1 of HVT079 and HVT096 genes from the HVT genome to generate the mutant HVT-*ΔvNr-13* to gain insights into its functional roles. Overall, both wild type HVT and HVT-*ΔvNr-13* showed similar growth kinetics; however, at early time points, HVT-*ΔvNr-13* showed lower growth of 1.3- to 1.7-fold of cell-associated virus and 3- to 6.2- fold of cell-free virus. HVT *vNr-13* localized in mitochondria, endoplasmic reticulum (ER) and nuclear membrane, and demonstrated to disrupt mitochondrial network morphology, while substantially restored during HVT-*ΔvNr-13* infection than wild type HVT. IncuCyte® S3 real time apoptosis monitoring demonstrated that *vNr-13* unequivocally involved in the apoptosis inhibition, and it is associated with increase of PFU, especially at serum-free conditions of later stages of viral replication cycle. Furthermore, HVT blocks apoptosis in infected cells, but activates apoptosis in bystander cells.

3) TARGETED GENE DELETION/ACTIVATION IN MAREK'S DISEASE VIRUS-TRANSFORMED CELL LINES USING CRISPR/CAS9 AND CRISPRa SYSTEMS

Poornima Roy, Venugopal Nair, and Yongxiu Yao
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Lymphoblastoid cell lines derived from lymphomas induced by Marek's disease virus (MDV) are valuable for interrogating viral and host gene functions as well as identification of molecular determinants associated with the maintenance of MDV latency and lytic switch. Previously we have established an efficient pipeline for *in situ* CRISPR editing of the MDV genome in lymphoma-derived cell lines. Using this approach, we have demonstrated viral genes such as pp38 and MDV-miR-M4 are not essential for maintaining the transformed phenotype. Meq protein, which is expressed both in lytic and latent infections, is the most important viral gene associated with MD oncogenicity. Yet the role of Meq in maintenance of MDV transformed phenotype is still unclear. Using CRISPR dropout assay which involves Meq deletion with CRISPR/Cas9 system and quantification of live/dead cell population by FACS analysis, we showed that deletion of Meq resulted in cell death demonstrating the essential role of Meq in transformation.

pp38, another important gene in MDV pathogenesis, is a unique phosphoprotein silenced in latency and activated during lytic switch. The induction of reactivation correlates with a marked increase in pp38 expression, and this expression has been used to discern lytically-infected from latently infected lymphocytes. We employed CRISPRa technology for targeted activation of pp38 in MDV cell lines to investigate its role in inducing lytic infection. The triggering of MDV lytic replication by pp38 activation will be presented.

4) AN ACTIVATOR MICRORNA : CHARACTERIZATION IN MAREK'S DISEASE VIRUS

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How GaHV-2 is able to tightly regulate its gene expression, to invade the host and to produce lymphoma before the death of the animal, is far from being fully described. A bicistronic transcript encompassing ICP22 and US10 ORFs was recently described. This peculiar transcript was shown to be targeted by three miRNAs. One of them, MDV-miR-M4, was unexpectedly shown to increase the amount of bicistronic transcript and was shown to "turn on" the expression of the ICP22 protein. It is still not known how miR4 binds to its target to enhance gene expression.

Using bioinformatics, primary and secondary RNA structures were described within the mRNA. From this analysis, the main working hypothesis were addressed through two questions : (1) Do specific RNA motifs have an impact on the translation of both ORF localized in the bicistronic transcript? (2) Is it possible to interfere with these motifs through miRNA-mRNA interaction?

To investigate these questions two fluorescent reporters were used to monitor the expression of the genes both in the presence and in the absence of miRNA expression. Next to this, the half-life of the transcripts were measured both in the presence and in the absence of miRNA expression.The obtained results shed light at a secondary structure belonging to the messenger RNA, localized at the microRNA binding site. This hairpin structure was revealed to have a prominent role at both the transcriptional and translational levels. The microRNA was also shown to play a substantial role which was both direct and indirect.

5) ADPONECTIN AND ITS RECRPTOR GENES EXPRESSION IN RESPONSE TO MDV INFECTION OF WHITE LEGHORNS

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Marek's disease virus (MDV) causes T cell lymphoma in susceptible chicken. MDV-infection is also related to an imbalance of the lipid metabolism in infected chickens. Adiponectin is circulatory cytokine secreted from adipose tissue and exerts critical metabolic functions. Although the associations between adiponectin and diseases have been reported, little is known about the relationship between MDV infection and adiponectin. Here, we explored phenotypic characteristics, plasma lipoprotein levels, adiponectin and the levels of its receptors in chicken post MDV infection. Our Data showed that MDV infection induced body weight loss in all the experimental birds. The concentrations of total cholesterol and HDL were lower after the infection. However, the infection did not affect adiponectin circulating levels in plasma. In abdominal fat, there was no significant difference of *adiponectin* mRNA level but a significant decrease of protein expression in the infected compared to the non-infected susceptible chickens. Moreover, we observed that the *adipoR1* and *adipoR2* at both mRNA and protein levels were decreased in susceptible chickens post MDV infection. In spleen, MDV infection significantly reduced the adiponectin mRNA expression but increased the protein in susceptible chickens. Furthermore, MDV infection decreased both *adipoR1* mRNA expression and protein levels. Also interestingly, the *adipoR1* mRNA expression level was significantly increased in susceptible chickens in liver post MDV infection. Taken together, our data provided interesting insights of adiponectin metabolism in chickens in association with MDV infection, which would help us advance the understanding on lipid metabolism in response to avian herpesvirus infection.

6) MAREK'S DISEASE VIRUS ONCOGENE MEQ EXPRESSION IN LATENTLY INFECTED CELLS IN VACCINATED AND UNVACCINATED HOSTS

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It is unknown how Marek's Disease (MD) vaccines prevent the appearance of lymphomas without eliminating virulent virus from the host, though the existing evidence suggests that mechanisms other than adaptive immunity likely plays a significant role. We hypothesized that vaccination effects MD pathogenesis at some point between lytic replication and tumorigenesis: latency. We previously reported a fully virulent recombinant MDV that expresses GFP under the Meq promoter (G2M). To test the effects of vaccination on latent infection dynamics, we investigated Meq expression and virus load in PBMCs from vaccinated and unvaccinated birds over a 5-week period after challenge with G2M. In unvaccinated birds, Meq positive CD4+ T-cells appeared within the first week, peaked in frequency at 2 weeks, and decreased to low stable levels by 3 weeks as we previously reported. Vaccinated birds showed significantly lower frequencies of Meq positive CD4+ T-cells in PBMCs compared to unvaccinated birds in the first week of infection. However, Meq negative T-cell population from both vaccinated and unvaccinated birds did have detectable levels of viral genome via qPCR. The difference in viral genome copy numbers was not statistically significant between these groups of birds. This indicates that vaccination suppresses the appearance of Meq-expressing cells early after infection without affecting latency in non-Meq-expressing cells. Identifying the unique features of these Meq positive compared to Meq negative latently infected cells may be key to uncovering the vaccine mechanism of protection. Currently we are working to positively identify latently infected cells that do not express Meq.

Session 3: Pathogenesis and Virus-Cell Interactions Panel 1

Moderated by: Dr. Mark Parcells

1) THE ROLE OF HOST GENETIC RESISTANCE ON MAREK'S DISEASE VIRUS TRANSMISSION

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Marek's disease (MD) is currently controlled through biosecurity, widespread vaccination, and selection for genetic resistance. Marek's disease virus (MDV) field strains have undergone multiple shifts of increased virulence that required introduction of new vaccines. This cycle of virus evolution followed by introduction of new vaccines is not sustainable in this large, expanding, and highly concentrated industry. In this study, we examined the potential role of genetic resistance in reducing quantity and duration of viral transmission, with the goal of reducing environmental virus load and thus increase the efficacy of existing and future control measures. We used a donor-recipient challenge model to determine when, how much, and how long MDV was transmitted. Donor birds differed by genetic resistance (ADOL Line 6, Line 7, or Hy-Line W36) whereas recipient birds were all highly susceptible (ADOL Line 15x7). Donor birds were transferred every 2 days to naïve recipient birds between days 10-20 post-challenge. Our results indicated that host genetics has an effect on virus load in feathers of donor birds, but did not have a significant effect on delaying the initiation of virus transmission or subsequent pathogenicity in recipient birds. Compared to our past studies, these results demonstrate that host genetics has a much smaller effect on virus transmission compared to MD vaccination.

2) VACCINE-DRIVEN EVOLUTION OF VIRULENCE: THE CASE STUDY OF MAREK'S DISEASE VIRUS

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Vaccination has the potential to drive pathogen evolution. In the case of Marek's disease virus, an economically important chicken pathogen, this evolution has manifested as an increase in pathogen virulence. Using a data-informed mathematical model of Marek's disease virus transmission on farms, we show that correlations between virus shedding rates and virulence cause less virulent v isolates of the virus to be selectively favored over more virulent vv and vv+ isolates in nonvaccinated chickens, but hypervirulent vv+ isolates to be selectively favored over low and moderate virulence v and vv isolates in vaccinated chickens, for all three licensed Marek's disease vaccines. Despite 50 years of nearly universal vaccination, however, low and middle virulent virus isolates still co-circulate with hyper virulent isolates, and it is unclear why. By expanding the model to describe virus dynamics on many farms over time, we show that the model can explain the observed historical increase in virulence, but it cannot explain the long-term maintenance of diversity in virulence. We further extend the model to explore several hypotheses for why variation is maintained, and we conclude that the best explanation is that the relationship between pathotype and virus shedding in vaccinated hosts seen previously may not hold for currently circulating virus isolates and currently used vaccines. This model, which allows for interactions between vaccine types and virus pathotypes, predicts that mean virulence will decrease over time and that vaccine protection may be restored -- a pattern preliminarily seen in pathotype data.

3) PATHOGENICITY OF VERY VIRULENT PLUS MAREK'S DISEASE VIRUS WITH MODIFIED VIRULENCE-ASSOCIATED GENES

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Over the past decades, Marek's disease virus (MDV) has evolved toward greater levels of virulence, and three pathotypes are currently recognized virulent, very virulent and very virulent plus. Since current vaccines fail to provide sterilizing immunity and the implementation of different vaccines parallels the evolution of the higher virulent pathotypes, the specific mutations associated with these changes has been the subject of comparative genomic studies. These studies have mainly focus on the genes within the repeat long (namely meq) and its role in the evolution of MDV toward greater virulence. We recently characterized 70 MDV genomes with known virulence by complete or targeted DNA sequencing and identified single nucleotide polymorphisms (SNPs) that showed association with virulence in 8 genes UL22, UL36, UL37, UL41, UL43, R-LORF8, R-LORF7, and ICP4. Using the BAC containing the genome of very virulent plus MDV strain 686, specific base pair changes were introduced in loss-of-function experiments to investigate the effects of these SNPs in virulence. Nine SNPs in eight genes within a single genome were introduced and verified by targeted sequencing. The pathogenicity of the reconstituted virus from the modified 686-BAC recombinants and that of the 686-BAC-derived parental virus will be discussed.

4) HYPOXIC MEDIATED LATENT AND LYTIC SWITCH OF MAREK'S DISEASE VIRUS

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Marek disease virus (MDV) is an oncogenic alphaherpesvirus, characterized by rapid onset of T-cell tumours in chickens. Latency is distinct feature of MDV, where the virus silently persists in the infected/transformed cells with occasional reactivation to lytic cycle. However, the viral mechanisms/factors that regulate MDV latency and lytic switch are poorly understood. It has been known that tumours grow under poorly oxygenated (0.1 to 1% oxygen, hypoxia) conditions *in vivo* but it is still remains unknown on the role of *in vitro* hypoxia on MDV latency and/or lytic switch. Thus, role of hypoxia (1% O_2) on MDV latency and lytic replication was elucidated in MDV transformed 4839K_GFP and MSB1 lymphoblastoid cell lines in comparison to Normoxia (21% O_2). According to flow cytometric analyses, in 4839K cells at 24 hours, there was significant differences in the expression of GFP positive cells were observed between hypoxic and normoxic conditions. In MSB1 cells, significant difference in the percentage of PP38 (latent gene) positive cells were observed between hypoxic and normoxic conditions with flow cytometric and qPCR analyses. Furthermore, according to transcriptomic analyses most viral latent genes were highly upregulated. Thus, the results of hypoxic mediated latent and lytic switch of MDV are important to understand role in replication cycle and pathogenesis. In the future, the higher fold change viral lytic genes role will be evaluated in detail by CRISPR/Cas9 approach to understand the *in vivo* significance in MDV pathogenesis.

5) EPIGENETIC SILENCING OF A HOST MICRORNA PLAYS A ROLE IN THE PROLIFERATION OF LYMPHOBLASTOID CELL LINE INFECTED WITH MAREK'S DISEASE VIRUS

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During latency, herpesvirus infection results in the establishment of a dormant state in which a restricted set of viral genes are expressed. This is associated with extensive DNA methylation in non-expressed genes. Together with these alterations of the viral genome, several host genes undergo epigenetic silencing during latency. These epigenetic dysregulations of cellular genes might be involved in the development of cancer. In this context, Marek's Disease Virus (MDV) was shown to impair the expression of several cellular microRNAs (miRNA) in chicken. We decided to focus on miR-126, a host miRNA considered as a tumor suppressor through signaling pathways controlling cell proliferation and survival. Our objectives were to analyze the cause and the impact of miR-126 silencing during MDV infection. The cellular miRNA was found to be repressed at key steps of the viral infection. In order to determine whether miR-126 low expression level was associated with specific epigenetic signatures, DNA methylation patterns were established in the miR-126 gene promoter. Repression was found to be associated with hypermethylation at a CpG island located in the miR-126 host gene. To determine the role of miR-126 repression during MDV transformation, we analyzed the impact of miR-126 restoration. A strategy was developed to conditionally overexpress miR-126 and control miRNAs in transformed CD4+ T cells propagated from MD lymphoma. This functional assay showed that miR-126 restoration specifically blocked cell proliferation. Ongoing analysis aims at identifying the panel of miR-126 target genes involved in the control of the cell proliferation.

6) ASSESSMENT OF THE ROLE OF IRG1 AND ITACONATE ON MAREK'S DISEASE VIRUS (MDV) INFECTION

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Marek's Disease (MD) is a pathology of chickens characterized by paralysis, immune suppression, and the induction of T-cell lymphomas. MD is caused by the *Alphaherpesvirus*, Marek's Disease Virus (MDV). In 2011, immunoresponsive gene 1 (IRG1) was identified through high-throughput screening and QTL analysis as a key factor in MDV susceptibility and resistance, but no mechanistic studies have corroborated this finding. IRG1, is induced in macrophages during inflammation and generates itaconic acid (ITA). ITA has multiple functions mediated by its conjugation to cellular, viral, and bacterial proteins. For instance, ITA inhibits succinyl dehydrogenase, causing a shift in metabolism from the tricarboxylic acid cycle to glycolysis. ITA is conjugated onto several viral proteins, inhibiting their function. Secreted ITA has antimicrobial activity through its inhibition of bacterial metabolism. ITA also regulates the levels of nuclear erythroid related factor 2 (NRF2), a transcription factor that regulates anti-inflammatory and anti-oxidative genes. We have examined IRG1 expression during MDV pathogenic (rMd5) and vaccine (rMd5ΔMeq) virus replication, *in vivo*. We found that IRG1 was induced at 4 dpi by rMD5 and then downregulated, while IRG1 was significantly induced at 7, 14 and 21 dpi during rMd5ΔMeq replication. Using a cell-permeable derivative of ITA (4-octyl itaconate, 4OI), we found that this chemical inhibits MDV replication in CEF and spleen cells. We have also found that overexpression of IRG1 in HD11 cells induces the secretion of a factor that also inhibits MDV replication. Together, our data provide some mechanistic understanding of how IRG1 may be important to MDV resistance.

Session 3: Pathogenesis and Virus-Cell Interactions Panel 2

Moderated by: Dr. Benedikt Kaufer

1) EVOLUTIONARY CHANGES IN THE MAJOR VIRUS-ENCODED ONCOGENE DETERMINE PATHOGENICITY AND SHEDDING OF MAREK'S DISEASE VIRUS

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Marek's disease virus (MDV) constantly evolves towards a greater virulence resulting in a broad range of pathotypes. Despite many years of research, the mechanism and proteins that are driving this evolution of more virulent strains remain mostly unknown. Changes in the major oncogene *meq* correlate with the increase of virulence; however, it remains elusive if they provide an evolutionary advantage for the virus. To determine if the changes in *meq* contribute to the increased virulence, we replaced the meq gene in the very virulent RB-1B MDV strain with *meq* isoforms from different pathotypes and tested them *in vitro* and *in vivo*. Replacement of *meq* isoforms only had a minor effect on virus replication. Strikingly, insertion of *meq* isoforms from lower virulent pathotypes completely abrogated or dramatically reduced tumorigenesis. Insertion of vv+ meq isoforms did not increase virulence, indicating that other changes in the viral genome are required to achieve the virulence of vv+ strains. In addition, we assessed if meq isoforms also contribute to breaking vaccine protection and enhance shedding in vaccinated animals. The vv and vv+ meq isoforms allowed the virus to efficiently overcome vaccine protection and enhanced shedding of the virus into the environment. Our study provides the first experimental evidence that evolutionary changes in the *meq* gene are critical for the increase in pathogenesis and levels of viral shedding of this deadly pathogen.

2) VALIDATING THE ROLE OF IKAROS AS A CANCER DRIVER GENE FOR MAREK'S DISEASE

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Marek's disease (MD) is characterized by the rapid onset of lymphomas and nerve enlargement following infection of susceptible chickens with pathogenic Marek's disease virus (MDV). Despite the success of widespread MD vaccination, a number of key questions remain unanswered that are needed to prevent the repeated emergence of more virulent MDV field strains and achieve sustainable MD control. It is known that MDV Meq is necessary but not sufficient for tumor formation. Therefore, we hypothesized that somatic mutations in specific chicken (host) genes are necessary, which is similar to what is observed for human cancers. Using multiple genomic screens on MD tumors, we identified several candidate cancer driver genes including Ikaros (IKZF1), the master regulator of lymphocyte development, which was somatically mutated in key Zn-finger domains needed for DNA binding. To validate that Ikaros is an MD driver gene, we engineered recombinant MDVs to express either wild-type or mutated alleles of Ikaros. We hypothesized that the expression of the mutated but not the wild-type allele would enhance transformation of infected CD4 T cells, i.e., MDV Meq provides anti-apoptotic function while mutated Ikaros would enable unregulated cell growth. Results from live bird challenges with these recombinant MDVs confirmed this model. In conclusion, this experiment validates that Ikaros is the first MD driver gene to be reported and provides critical insights on MD pathogenesis.

3) US3 SERINE/THREONINE PROTEIN KINASE FROM MDV-1, MDV-2, AND HVT DIFFERENTIALLY REGULATE VIRAL REPLICATION AND PATHOGENESIS

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Gallid alphaherpesvirus 2 (GaHV-2), commonly known as Marek's disease virus type 1 (MDV-1), is an oncogenic avian alphaherpesvirus, and along with its close relatives *Gallid alphaherpesvirus* 3 (GaHV-3) or MDV-2 and *Meleagrid alphaherpesvirus* 1 (MeHV-1) or turkey herpesvirus (HVT), belongs to the *Mardivirus* genus. We and others previously showed that MDV-1 US3 protein kinase plays an important role in viral replication. To further study the role of US3 in MDV replication and pathogenicity, we generated an MDV-1 US3-null virus and chimeric viruses by replacing MDV-1 US3 with MDV-2 or HVT US3. Using MD as a natural virus-host model, we showed that both MDV-2 and HVT US3 partially rescued the growth deficiency of MDV-1 US3-null virus *in vitro*. Similarly, MDV-2 and HVT US3 could also partially rescue the replication deficiency of MDV-1 US3-null virus in spleen and thymus, as determined by immunohistochemistry analysis of MDV-1 pp38 protein and genome copy number measurement. Importantly, using immunohistochemistry and dual immunofluorescence assays, we found MDV-2 US3, but not HVT US3, fully compensated MDV-1 US3 regulation of MDV-1 replication in bursal B lymphocytes. In addition, deletion of MDV-1 US3 attenuated the virus resulting in higher survival rate and lower MDV specific tumor incidence, which could be partially compensated by MDV-2 and HVT US3. In conclusion, our study provides the first comparative analysis of US3 from MDV-1, MDV-2 and HVT in regulating viral replication and pathogenesis.

4) MAREK'S DISEASE VIRUS US3 PROTEIN KINASE PHOSPHORYLATES CHICKEN HDAC1 AND 2 TO REGULATE VIRAL REPLICATION

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Marek's disease virus (MDV) is a potent oncogenic alphaherpesvirus that elicits a rapid onset of malignant T-cell lymphomas in chickens. Three closely related virus species, including GaHV-2 (MDV-1), GaHV-3 (MDV-2) and MeHV-1 (HVT), have been identified and all encode a US3 protein kinase. MDV-1 US3 is important for efficient virus growth *in vitro* and was shown to phosphorylate MDV-1 pp38 and Meq, as well as chicken cAMP response element binding (CREB) protein. In this study, we identified chicken histone deacetylase 1 (chHDAC1) as a common US3 substrate for US3 from MDV-1, MDV-2 and HVT, while only US3 of MDV-1 and MDV-2 phosphorylate chHDAC2. We further determined that US3 of MDV-1 and HVT phosphorylate chHDAC1 at serine 406 (S406), while MDV-2 US3 phosphorylates S406, S410, and S415. In addition, MDV-1 US3 phosphorylates chHDAC2 at S407, while MDV-2 US3 targets S407 and S411. Furthermore, biochemical studies show that MDV US3 mediated phosphorylation of chHDAC1 and 2 affect their stability, transcriptional regulation activity, and interaction network. We also demonstrated that US3 from MDV-1, MDV-2 and HVT interacts with chHDAC1 and 2 in a kinase activity dependent manner. Using a class I HDAC specific inhibitor, we showed that MDV-1 US3 mediated phosphorylation of chHDAC1 and 2 is involved in regulation of viral replication. Here, we will present novel substrates for MDV US3 and discuss the role of MDV US3 in MDV replication.

5) SEQUENTIAL INTERACTIONS OF MEQ PROTEINS WITH POLYCOMB REPRESSIVE COMPLEX PROTEINS IN MAREK'S DISEASE VIRUS LATENCY

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Marek's disease virus (MDV) causes a rapid onset of T-cell lymphomas in chickens. T-cell lymphomas are formed during the latency of MDV. T-cell lymphomagenesis also requires the expression of Meq, an MDV-encoded basic leucine zipper transcription factor protein, to transform T-lymphocytes. Both lytic and latently-infected lymphocytes express a full-length, unspliced form of the Meq protein *in vivo*, but during latency and in transformed T-cells, MDV also expresses splice variants of Meq (Meq/vIL8 and Meq/vIL8 exon3). MDV mediates T-cell transformation through the interactions of Meq proteins with various cellular proteins including CtBP, c-Jun, etc. We hypothesize that full length Meq protein co-localizes with EZH2, a histone 3, lysine 27 trimethylase (H3K27me3). EZH2 is the enzymatic part of polycomb repressive complex 2 (PRC2). Spliced forms of Meq interact preferentially with Bmi-1, the DNA-binding component of polycomb repressive complex 1 (PRC1) primarily via exon 2 of Meq/vIL8. This binding results in translocation to the nucleolus and decreased mobility of Bmi-1 to that of Meq/vIL8. In other systems, PRC1 further silences genetic loci through the mono-ubiquitination of histone 2A at position 119. Our work suggests that as latency is established, there is a sequential recruitment of PRC2 and PRC1 to the MDV genome, as well as to chicken chromosomal loci, by different forms of the Meq protein, and that these sequential interactions contribute to the efficiency of MDV genome silencing, as well as the transformation of latently-infected T-cells.

6) EVALUATION OF THE EFFECT OF MEQ ISOFORM ON MAREK'S DISEASE VIRUS (MDV) PATHOGENICITY

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Hallmarks of Marek's disease virus (MDV) infection of chickens are paralysis, immune suppression, and the rapid induction of T-lymphomas. The transformation of T-cells by MDV requires expression of the oncoprotein Meq. Marek's disease (MD) is currently controlled by vaccination using live-attenuated, apathogenic vaccines. These vaccines, however, do not elicit sterilizing immunity, which has contributed to the emergence of increasingly virulent field strains. Mutations identified in the Meq coding sequence largely correlate with this virulence evolution. The present study addresses the hypothesis that mutations in Meq have been selected by (1) the innate immune response elicited by host vaccination during the early lytic infection stage, and/or (2) changes in the Meq interactomes in latently infected T-cells. Strain RB-1B-based recombinant-MDVs expressing Meq isoforms from JM102, CVI988-S (339 aa form), CVI988-L (399 aa form), the RB-1B parent virus, strain 617A, a triple mutant having P153Q, P176A, and P217A C-terminus mutations, and the N-strain, having the combination of these mutations were constructed. To address our hypothesis, groups of unvaccinated, and *in ovo* HVT-, and HVT/SB1-vaccinated specific pathogen-free chickens were challenged at hatch with 2000 PFU of each of these viruses. We are examining changes in virus replication, mortality, and tumor incidence of each treatment, as well as changes in vaccine efficacy after challenge with these recombinant viruses. In addition, we are examining the interactomes of these Meq isoforms in the context of early lytic infection, primary lymphomas, and cell lines established from lymphomas induced by these recombinant viruses.

A full list of MDAH-2021 participants will be available shortly.
Thank you.