IVIS2023

KRUGER PARK | SOUTH AFRICA

13TH INTERNATIONAL VETERINARY IMMUNOLOGY SYMPOSIUM

17-21 NOVEMBER 2023

PROGRAMME & ABSTRACTS

www.ivis2023.org

CSIRO

Australia's National Science Agency

Delivering advanced solutions in host-response, data science and vaccine development

CSIRO has a strong record of delivering breakthrough innovations and technologies with real-world impact.

We use a cross-disciplinary, collaborative approach to develop diagnostics and vaccines for zoonotic and livestock diseases.

Our scientists have wide-ranging capabilities, including disease expertise, data analytics, modelling, social science, immunology and biology.

Access to world class facilities such as the Australian Centre for Disease Preparedness with BSL2, 3 and 4 laboratories and animal rooms allows us to address current challenges from pathogen discovery to the delivery of novel tools for disease control.

csiro.au

Table of Contents

Local Organizing Committee

Prof. Sven Parsons (South Africa) – Chairperson
Dr Claire Rogel-Caillard (France) – Co-Chair
Dr Yolandy Lemmer (South Africa)
Dr Alejandra V. Capozzo (Argentina)
Dr Crystal Loving (USA)
Dr Eva Wattrang (Sweden)
Dr Vish Nene (Kenya)

Scientific Programme Committee

Dr Yolandy Lemmer (South Africa) – Chairperson
Dr Alejandra V. Capozzo (Argentina) – Co-Chair
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Prof. Alri Pretoria (South Africa)
Prof. Polly Roy (UK)
Dr Bettina Wagner (USA)
Prof. Yaofeng Zhao (China)

Contents

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Welcome

Dear fellow immunologists,

On behalf of the Organising Committee, it is our privilege to welcome you to the 13th International Veterinary Immunology Symposium (IVIS2023), hosted in the heart of one of Africa's iconic game reserves – the Kruger National Park, South Africa.

This is the first time this symposium is hosted in Southern Africa, which therefore creates an opportunity for meeting colleagues from Africa and the rest of the globe on African soil. You will be able to experience some of the African culture, tourism opportunities and local cuisine!

The Kruger Park and surrounding rural areas are emblematic of both the opportunities and threats that co-exist at the interface of human societies, their domestic animals, free-living wildlife, and the environments that they inhabit. In a context of global change, sustainably improved livestock systems and disease control are essential for not only the commercial livestock trade, but also for subsistence farming, food safety and security, human wellbeing, and the biodiversity conservation of ecologically and economically important ecosystems. In this spirit, the IVIS2023 will address the challenging research issues raised for veterinary science in a One World – One Health perspective.

The IVIS2023 will also bring together experts to share most exciting results and insights in the field of veterinary science and immunology, including biological mechanisms underlying host-pathogen interactions and specificities, vaccine and therapeutic strategies, innate and protective immunity, disease diagnosis and control, reagent and methodological developments. This will be an opportunity for delegates from all involved sectors to engage with leaders in the field and network in the comfort of a state-of-the-art conference facility.

We want to thank the Organising Committee for all their hard work and time to organize the symposium. The organizing team made a huge effort to "pull out all the stops" to make this a truly memorable event.

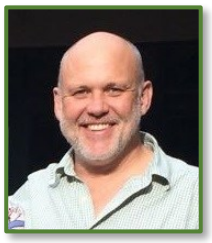
May we also take this opportunity to thank the scientific committee comprising both local and international members for evaluating abstracts and selecting papers for oral presentations and posters.

Last but not least, a big word of thanks to all our sponsors and exhibitors for contributing financially towards the symposium.

On behalf of the Organising Committee, who put in a lot of time apart from their normal daily jobs to organize the IVIS2023, we wish you a memorable congress, both scientifically and socially – enjoy your stay in South Africa!

Sven Parsons (South Africa) & Dr Claire Rogel-Gaillard (France)

Symposium Co-chairs



With appreciation to our Sponsors

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Quality Antibodies for Quality Research

The Scientific Group

UKRI Biotechnology and Biological Sciences Research Council

VR

About the Veterinary Immunology Committee (VIC)

The VIC Committee promotes and coordinates the interests of the international veterinary immunology community.

What is the VIC?

The Veterinary Immunology Committee (VIC) promotes and coordinates the interests of the international veterinary immunology community. A notable feature of veterinary immunology is its diversity – it encompasses a wide range of domesticated and wildlife animal hosts which are affected by disparate pathogens and immunological conditions.

Sub-committees and working groups with a specialised focus are established from time to time to assist VIC.

The Veterinary Immunology Committee is composed of 5-8 veterinary immunologists, co-opted with the aim of achieving equitable representation for different regions of the world. There is no formal election process for membership. A Chairperson and members are selected as needed at VIC meetings held at each International Veterinary Immunology Symposium. Members usually remain in office for two succeeding three-year terms.

Field of action

As a scientific discipline, veterinary immunology traverses topics ranging from fundamental studies on how the immune system functions to more applied areas such as production of vaccines and clinical applications of immunology. The main activities of VIC include:

- Promote veterinary immunology
- Assist with the planning and funding of International Veterinary Immunology Symposia (IVIS) – these are held every three years as satellite meetings of the International Symposium of Immunology;
- Coordinate databases containing information of interest to veterinary immunologists;
- Sponsor and support regional workshops and conferences;
- Coordinate the development of a toolkit of immunological reagents suitable for use in animals of veterinary interest;
- Administer the Distinguished Veterinary Immunologist Award.

Committee Members

- Claire Rogel-Gaillard (France, Chairperson)
- Eva Wattrang (Sweden, VIC Treasurer)
- Alejandra Capozzo (Argentina, AVI, ALAI)
- Gerald Chege (South Africa, FAIS)
- Gary Entrican (UK, Past-Chair)
- John Hammond (UK, VIC MHC Chair)
- Jayne Hope (UK, VIC Toolkit Chair)
- Gregers Jungersen (Denmark, EVIG Chair, EFIS)
- Chieko Kai (Japan, FIMSA)
- Crystal Loving (USA, AAVI, IVIS 2019 Chair)
- Isobel Kinney Ferreira de Miranda Santos (Brazil, ALAI)
- Sven Parsons (South Africa, IVIS 2023 Chair)

CSIRO

Delivering advanced solutions in host-response, data science, and vaccine development.

CSIRO has a strong record of delivering breakthrough innovations and technologies.

At the Australian Centre for Disease Preparedness (ACDP), we have provided research and diagnostic outputs in livestock health for nearly 40 years. This has resulted in the successful deployment of a vaccine for prophylaxis against Hendra virus infection. One focus area is developing vaccine platforms addressing the challenges where vaccines are not available or not optimal.

In addition, we now have a standalone research program focusing on immune resilience.

The facility hosts 10 World Organisation for Animal Health Reference Laboratories, demonstrating our strong commitment to diagnostic services and research into improved diagnostics, and expanding into platform technologies and bioinformatics.

We realise that deploying novel technologies is a complex process where human behaviour needs to be considered. The impact often depends on the broader epidemiology of diseases and local circumstances. Therefore, we believe in a trans-disciplinary approach to solving problems and threats by involving other disciplines, such as social sciences and disease modelling.

At ACDP, we take pride in our BSL2, 3 and 4 laboratories and animal rooms that allow us to address current challenges from pathogen discovery and vaccine testing to the delivery of novel tools for disease control.

We believe a collaborative approach by bringing teams together will deliver real-life impacts. We are excited to work with you to make a positive impact together.

CSIRO aims to deliver a better future for everyone: our people, our planet and our economy.
https://www.csiro.au/en/



MABTECH

ELISpot, FluoroSpot, and ELISA

We've got kits for over 20 different target species (here we show the ones used in veterinary research).

Cow

IFN-γ
IgG
IL-2
IL-4
IL-5
IL-8 (CXCL8)
IL-17A
TNF-α

Horse

IFN-γ
IgG
IL-10
IL-17A

Sheep

IFN-γ
IL-2
IL-4
IL-5
IL-17A

Pig

IFN-γ
IgG
IL-2
IL-17A
Perforin
TNF-α

Goat

IFN-γ
IL-4
IL-5
IL-17A

Dog

IFN-γ
IL-8 (CXCL8)
IL-17A

Cat

IFN-γ

Chicken

IFN-γ

Salmon

IFN-γ

Buffalo

IFN-γ

Llama

IFN-γ

Alpaca

IFN-γ

Lion

IFN-γ

Cheetah

IFN-γ

Rhino

IFN-γ

Visit our website for the latest update

Welcome to South Africa

Despite horror stories of sky-high crime rates, most people visit South Africa without incident; be careful, but don't be paranoid. This is not to underestimate the issue – crime is probably the most serious problem facing the country. However, once you realize that crime is disproportionately concentrated in the poor African and coloured townships, the scale becomes less terrifying. Violent crime is a particular problem not just in the townships but also in Johannesburg, where the dangers are the worst in the country.

If you fall victim to a mugging, you should take very seriously the usual advice not to resist and do as you're told. The chances of this happening can be greatly minimized by using common sense and following a few simple rules:

- At night, stay away from dark, isolated areas. It is always better to explore in groups and to stick to well-lit, busy streets.
- Avoid mass public transport systems in cities.
- Politely refuse anything you're offered by a 'friendly' stranger.
- Try not to look like a tourist — keep things like cameras and maps tucked away.
- Don't wear expensive items or flash around money and electronics.
- Distribute valuables across different pockets and consider having a decoy wallet with a few Rand in it to fool muggers.
- When driving, keep the doors locked and windows up. Don't pick up hitchhikers or stop for motorists who appear to be broken down — these are ploys commonly used by robbers and carjackers.

Kruger National Park

The Kruger National Park is one of South Africa's icons and perhaps its greatest tourist attraction. The Park lies in the north-eastern corner of the country, at the foot of a great escarpment, in an area known as the Lowveld. It borders Mozambique in the east and Zimbabwe in the north and is home to a huge diversity of plant, bird and animal life – said to be more diverse than in any other conservation area in Africa.

General Malaria Information

Malaria is a word many people associate with game parks in Africa. However, only two of the South African National Parks are in a malaria risk area and they are the Kruger National Park and Mapungubwe National Park, although at both these venues the risk is usually low. Historically there have been incidences of malaria in other parks, but then there are recorded incidences of malaria from urban Europe and other non-risk areas. But to all intents and purposes Kruger is the only malaria risk park in the SANParks'set-up.

Anti-malaria prophylactics are thus recommended by visitors for Kruger. The highest risk period is between December and April (end of the rainy season). A 24-hour malaria hotline is available on +27 (0)82 234 1800 to give detailed explanation on risk and advice on precautionary measures. Visitors wishing to make use of prophylactics should consult a knowledgeable medical practitioner or recognized travel clinic about recommended medication, as certain products cause nausea, hallucinations or other negative side effects with certain people.

Here are 10 interesting facts:

1. It's huge. Sprawled across some 20 000 square kilometres, it is larger than some countries.
2. The Park is 360km long and about 65km at its widest point.
3. It contains about 27 000 buffaloes, 12 000 elephants, 2 000 lions, 10 000 rhino and 2 000 leopards.
4. It has 21 tourist rest camps and 15 private safari lodges.
5. Established on 26 March 1898, the Park was originally called the Sabi Game Reserve. It was expanded over the years, finally achieving national status in 1926 when it was given its present name in honour of President Paul Kruger of the Transvaal Republic.
6. Colonel James Stevenson Hamilton was appointed as first warden of the reserve in 1902. A Scottish cavalry officer who had fought in the Anglo Boer War, his appointment proved to be an inspired choice. Over the next 44 years he toiled tirelessly in steering the Park towards the wildlife sanctuary it is today.
7. Of the 517 species of birds occurring in Kruger, 253 are residents, 117 are non-breeding residents and 147 are nomads.
8. It is home to 114 species of reptiles, including about 3 000 Nile crocodiles and various snakes of which the black mamba and puff adder are the most dangerous.

9. There is evidence of human occupation dating back 1.5 million years. South Africa's original inhabitants, the Bushmen, lived in a number of the caves and rocky shelters found in the area and left behind a legacy of Stone Age tools and artefacts as well as numerous rock paintings.
10. The Thulamela Iron Age Site, near Pafuri in the far north, is a stone walled site built approximately 450 – 500 years ago. Various artefacts unearthed on site, provide proof that the community was part of a dynamic trade network controlled by Arab merchants off the east coast of Africa during this period.

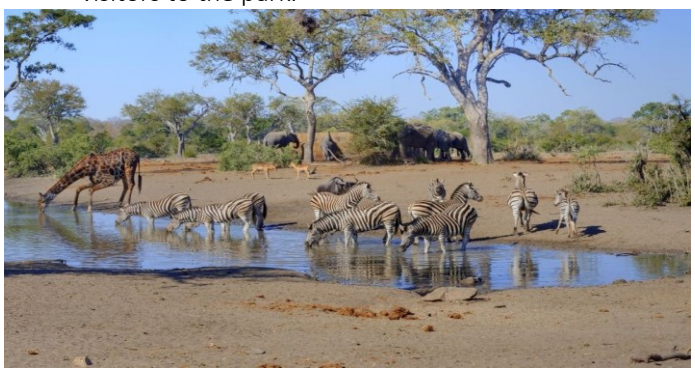
A leopard rests in a tree © Simon Eeman / Shutterstock

Kruger National Park Camp and Gate Hours

Camp and Gate entrances are closed after dark so please ensure that you have allowed sufficient travelling time to arrive at your rest camp before the stipulated closing time.

Take note

- The speed limit in the park is 50kph on tar and 40kph on gravel.
- Malaria treatment is essential prior to visiting the park.
- Do not litter, it is a hazard to the animals and is an offence.
- There are no television sets in the Park so that visitors can enjoy their surroundings, tourists that bring their own may not disturb other visitors to the park.



Is the Kruger dangerous?

Always remember that you're driving in the presence of wild animals. Stay in your car, keep your windows up unless you're after a furry passenger, and make sure you carefully follow the other park regulations.

Learn how to act around big game, particularly elephants: give them plenty of room, switch off your engine, stay quiet and never get between an elephant mother and her calf. If you spot any signs of aggression, such as an elephant kicking up dust, flapping its ears and trumpeting, back away slowly, as they can flip cars. If you follow these rules, you'll rarely get into trouble.

What shouldn't you miss?

A bush walk. This is one of the only opportunities you'll get to head off into the long grass on foot – accompanied by a professional guide, of course. You'll gain a deeper understanding of the park's flora and fauna, and there's always the possibility of bumping into big game.

If that sounds too adventurous, then opt instead for an organised game drive either early in the morning or late at night. You'll have the chance to spot nocturnal animals, including genets, civets and owls, which you wouldn't otherwise see (as self-driving is only permitted in daylight hours). To book, enquire at any of the main camps.

The south of the park is best for first-time visitors as it has the densest population of big game, and there are some drives here you definitely shouldn't miss. First, there's the route from Skukuza camp to Satara: watch the sunrise from the bird hide at Lake Panic, then head north via the southernmost baobab tree. Just before you hit Satara, turn onto the S100 – the park's legendary white lion is most often spotted here.

Eating Out

There are a couple of restaurants and cafes to eat at, including the Selati Station Grillhouse, which is well-worth having a look at.

The Grillhouse is set around the original Selati Express train which brought the first tourists to Kruger National Park way back in the 1920s. Enjoy an evening there soaking up some Kruger Park history!

Activities

- Game Drives (Night and Morning)
- Bush Breakfast and Braai (Barbeque)
- Golf
- Guided Bush Walks
- Stevenson Hamilton Memorial Library
- Wilderness Trail (Metsi Metsi)

If you want to go on a **safari tour** from the rest camp, you can book:

- Morning and afternoon game walks
- Sunrise and sunset game drives
- Bush breakfasts and bush braais (BBQs)

Kids Educational Programme

3 Swimming pools, two of these are in camp and for overnight residents only. The third is in a separate new day visitor's area, located downstream from the main camp.

These activities will ensure an exciting bush experience. All bookings as well as further details of these activities are available from the Skukuza reception.

Facilities

- Information at Reception 08h00 – 18h00
- Public telephone – All hours
- Post Office – 09h00 – 15h00
- Bank – 09h00-15h00
- Basic First Aid
- Restaurant - 07h00-09h00 breakfast, 12h00-14h00 lunch, 18h00-21h00 dinner
- Cafeteria
- Shop
- Emergency Road Services
- Garage with Workshop
- Petrol Station
- Laundromat 9 Hole Golf Course - 013 735 5543
- Auditorium and Conference Facilities
- Car Wash
- Limited DSTV Available in Luxury Units
- Avis Car rental – 013 735 5651

Other Facilities

- The Parks Shops
- Skukuza has a relatively large shop where a variety of items can be purchased including:
- Curios
- Safari and Ethnic clothing
- Binoculars, books, camera films and Videos on wildlife
- Magazines
- Meat, groceries, fresh milk, vegetables, and bread daily.
- Beverages: Spirits, Beer, Wine Cold Drinks, Fruit Juices and Mineral water
- Hardware (limited stock)
- Sweets and snacks
- Medicines (non-prescription)
- Stationary and stamps
- Braai wood and other accessories

Areas of special interest

In Skukuza camp

- Stevenson Hamilton Memorial Library
- Dog Graveyard
- Selati Restaurant – the old railway carriage has been converted into a restaurant and sports bar

Out of camp

- Kruger Tablets
- Lake Panic Bird Hide
- Nursery
- Stevenson Hamilton Memorial
- Granokop

Five Things to seek

- Fruit Bat
- Thick-tailed Bush Baby
- Warthog
- Spotted Hyena
- Purple-crested Lourie

Warnings

Please ensure that your stay is happy and safe by taking note of a few simple warnings. You will be sharing your stay with many exciting and unusual creatures but without knowledge some of them could be dangerous!

Bats, Spiders, Snakes, Scorpions, Malaria Zone

If you must walk around at night, please do not do so without a torch.

Remember: By feeding any wildlife, you are signing their death warrant as they become aggressive.

New England Animal Health

Supporting Livestock Vaccine Development to Advance Food Security

NEAH is a U.S. non-profit company created to fill an important need hindering animal science researchers. That is, to provide a reliable source of immunological tools (monoclonal and recombinant antibodies) not readily available for livestock vaccine research. While some reagents can be accessed commercially, the breadth of valuable research reagents created by this community is far greater but not easily accessed. Our mission is to provide those reagents with minimal commercial viability, at a low cost.

We work with the scientists and institutions that have created these immunological tools, through formal agreements, to make their reagents readily available to the research community. If you have such reagents, please consider joining us.

Researchers will have the advantage of all using quality tested reagents, often from the same lot. This makes data sharing and comparison much more robust.

Reagent Donors

Moredun CSIRO ILRI

Manufacturing

Green Mountain Antibodies ImmunoTools, GmbH

Strategic Partnership

New England Animal Health
North & South America

Pentlands Immunologics
Europe, Africa and Asia

A critical element to the success of this endeavor is to find the right partners. NEAH and Pentlands Immunologics (a subsidiary of The Moredun Group) have joined in creating a non-profit partnership to collaborate on the production and distribution of affordable immune reagents specific for the study of immune responses in ruminants (sheep, goat and cattle) and swine.

We endeavor to provide a reliable, affordable supply of reagents, reducing shipping costs by organizing distribution geographically.
Our research community drives what reagents are on offer, not profits.

Get Involved, visit https://neah.org for more information

Participant Information

Registration Information
Each participant at IVIS 2023 must register in person at the Registration Desk to collect a Symposium kit and badge before attending any of the sessions or events.

Registration Times
Friday 17 November: 07h00 – 16h00
Saturday 18 November: 07h00 – 08h00
Sunday 19 November: 07h00 – 08h00
Monday 20 November: 07h00 – 08h00

Badges
Identification badges are required for admission to all sessions, official functions, and social events of the symposium. Participants who lose their badges must report to the Registration Desk, presenting proof of identity.

Presenters, Chairs & Facilitators
All speakers are required to report to the Registration Area at least 90 minutes before their presentation to ensure that we have uploaded the correct presentation onto the presentation laptop in the auditorium.

Poster Presentations
Posters will be available for viewing at the back of the plenary venue for the duration of the symposium. Four dedicated Poster Sessions will be hosted (one per day) and presenters are requested to present their posters during their dedicated time slots. Posters may be setup from Friday 17 November at 14h00.

Contacts
Ms Corné Engelbrecht | +27 (0) 82 925 9241
Ms Melanie Pretorius | +27 (0) 82 410 1202
Ms Nthabiseng Letsoalo | +27 (0) 73 509 4012

On-site Symposium Support

Emergency Medical assistance and Paramedic Services
For assistance with any medical emergencies, please visit the Registration area. Medical procedures and medicine will be for the attendee's own account. For any medical emergencies, please contact +27 (0) 82 925 9241 during symposium hours.

Meals and Snacks
Meals and beverages will be provided to attendees as indicated in the programme, during symposium times.

Safety and Security
In the interest of personal safety and security, attendees should only display their identity tags in the symposium area premises and within the restricted symposium areas.

Lost property can be handed in at the Registration Desk. Any losses should be reported to the Symposium Secretariat. Although every effort will be made to retrieve lost personal belongings, the responsibility for securing his/her personal belongings remains that of each person attending the symposium.

Accommodation and Transport
IMPORTANT: Excluding sponsored participants, all accommodation and transport arrangements will be for your own account.

Dress Code
The suggested dress code for the symposium is business casual, but please do bring something warm along as the rooms will be air-conditioned.

Liability
Neither the Symposium Secretariat nor any of its contracted service providers will be responsible for the safety of articles of any kind brought into the Symposium facilities by attendees, whether registered or not, their agents, contractors, visitors and/or any other person/s whatsoever. The Symposium attendee shall indemnify and not hold the organisers and associates of the organisers and their subcontractors liable in respect of any cost, claims, demands and expenses as a result of any damage, loss or injury to any person howsoever caused as a result of any act or default of the Symposium Secretariat or a person representing the Symposium Secretariat, its contractors, or guests. In addition, the Symposium attendee shall take all necessary precautions to prevent any loss or damage to his/her property with special regard to mobile phones, carry or handbags and computing equipment.

Foreign Delegate Information

Climate in the Kruger
The Kruger has two distinct seasons, the dry winter from May to October, and the April. However, every day in the Kruger National Park is a one-of-a-kind safari adventure.

In November, in Kruger National Park, Minimum temperatures vary between 19 in the morning and 32 in the afternoon. Temperatures are really hot. The average rainfall is around 49 inches, for 22 days without rain.

Language
South Africa has eleven official languages: English, Afrikaans and nine ethnic languages, of which Zulu and Xhosa are the most widely spoken. While most South Africans can communicate in more than one language, English is the most commonly spoken and the language of official business and commerce.

Languages Spoken in Kruger National Park: English, Afrikaans, Ndebele, Xhosa, Zulu.

Electrical Supply
Electricity is supplied at 220/240v. Both square (UK) and round (RSA) wall plugs are used.

Gratuity Guide

The amount you tip in South Africa will depend on where you are and what type of services you're buying. As a general rule, expect to tip around 10% of the bill. If you get exceptionally good service, say thank you with a tip closer to 15-20%.

Useful Numbers For Tourist In Kruger National Park

- SANParks - +27 12 428 9111
- Police - 10111
- Fire Brigade - 10177
- Ambulance - 10177
- 24- Hour Emergency Call Centre No: +27 13 735 4325

Currency

South African currency is the South African rand (ZAR).

Card services such as Visa, MasterCard, American Express and Diners Club are also accepted.

Foreign currency exchanges can be made at most banks and Bureaux de Changes.

Taxes and Refunds Within Kruger National Park

All goods and services have a 15% Value added tax (VAT) included. In order to reclaim VAT on purchases the total value of purchases must be over R250,00. Visitors are able to reclaim VAT at their departure, providing they have all their necessary receipts (a tax invoice stating VAT paid, your passport number and your bank account details.)

It is always advisable to keep a copy of the VAT form as a record for any follow-up on the transaction. VAT claims usually can be made at all major border posts and airports.

Banking Hours in Kruger National Park

Normal banking hours are weekdays between 09:00 - 15:30 and Saturdays 09:00 - 11:00.

Foreign currency can be exchanged at most banks and Bureaux de Changes.

Credit cards

Major credit cards, such as MasterCard and Visa, are accepted throughout the country, in most hotels, restaurants, retail outlets and safari companies. However, shops in remote areas and service stations may only accept cash.

Drinking Water

Tap water throughout the country is safe to drink. Bottled mineral water is readily available in most shops and supermarkets, and at camps and lodges. Tourists travelling by road are advised to carry sufficient water at all times.

Distinguished Veterinary Immunologist Award

It is our great pleasure to announce that **Dr Joan Lunney** has been awarded the Distinguished Veterinary Immunologist (DVI) Award, supported by Zoetis and selected by the IUIS Veterinary Immunology Committee (VIC) for outstanding research on Porcine Immunology and distinguished Service to the Veterinary Immunology Community.

The selection committee was chaired by Prof. Paul Wood.

In 2023, Dr Joan Lunney is the 10th recipient of this award.

Previous Award Winners:

- 12th IVIS 2019 Professor Paul Wood
- 11th IVIS 2016 Professor Serge Muyldermans
- 10th IVIS 2013 Professor Cynthia Baldwin
- 9th IVIS 2010 Professor Doug Antczak
- 8th IVIS 2007 Dr John Butler
- 7th IVIS 2004 Professor Wendy Brown
- 6th IVIS 2001 Dr Travis McGuire
- 5th IVIS 1998 Dr Alan Husband
- 4th IVIS 1995 Dr Richard Binns

Dr Joan Lunney will receive the award in person at **IVIS2023** in South Africa.

We warmly congratulate Joan for receiving this award and are looking forward to meeting you at IVIS2023.

zoetis

Programme Overview

FRIDAY 17 NOVEMBER 2023

08:30 – 12:30	Workshop 1
12:30 – 16:30	Workshop 2
17:00 – 17:15	Welcome & Opening
17:15 – 18:15	Keynote 1
18:15 – 19:15	Keynote 2
19:30 – 21:30	Welcome Function

SATURDAY 18 NOVEMBER 2023

08:30 – 10:00	Session 1
10:00 – 10:30	Mid-morning Refreshments
10:30 – 12:00	Session 2
12:00 – 13:00	Poster Session 1
13:00 – 14:00	Lunch
14:15 – 15:15	Plenary 1
15:25 – 16:00	Session 3
16:00 – 16:30	Refreshment Break
17:10	Close of Day
18:30	Informal Boma Braai – limited to guests who RSVP'd

SUNDAY 19 NOVEMBER 2023

08:30 – 10:00	Workshop 3
10:00 – 10:30	Mid-morning Refreshments
10:30 – 12:00	Workshop 4 & Session 5
12:00 – 13:00	Poster Session 2
13:00 – 14:00	Lunch
14:00 – 15:00	Plenary 2
15:00 – 16:00	Distinguished Veterinary Immunologist
16:00	Close of Day 2
16:30	Game Drive – limited to 200 guests
19:00	Bush Dinner – if you are unable to go on the game drive, the rangers will pick you up at the conference centre at 7pm.

MONDAY 20 NOVEMBER 2023

08:30 – 10:00	Session 6
10:00 – 10:30	Mid-morning Refreshments
10:30 – 12:00	Session 7
12:00 – 13:00	Poster Session 3
13:00 – 14:00	Lunch
14:00 – 15:00	Plenary 3
15:00 – 16:00	Keynote 3
16:00 – 16:10	Short Break
16:10 – 17:00	Keynote 4
17:00	Closing Ceremony

TUESDAY 21 NOVEMBER 2023

10:00 – 10:00	Light brunch in the conference venue
11:00 – 12:30	Workshop 5
12:30	Close of Event

Programme Schedule by Day

Thursday 16 November

Venue	Nari
09h00	Full day STAR-IDAZ MEETING – closed event

Venue	Safari Lodge - Mondzo Meeting Room
18h30	VIC Committee Meeting

Day 1 - Friday 17 November

VENUE	VENUE 1 – NDLOPFU	VENUE 2 - INGWE/MHELEMBE		VENUE 3 - NDAU/NARI
08h00	Setup of Venue	Registration for Workshop 1 opens	08h00	Setup of Venue
08h30		**Workshop 1** Pushing boundaries in veterinary immunology - A new era of NGS, with single cell sequencing and spatial transcriptomics *(10X Bioinformatics)*	08h30	
09h00			09h00	
09h30			09h30	
10h00			10h00	
10h30		Refreshment Break (Workshop 1)	10h30	
11h00		**Workshop 1 Continues**	11h00	
11h30			11h30	**Registration for Workshop 2 opens**
12h00			12h00	
12h30		Lunch (Workshop 1)	12h30	**Novel approaches to the development of veterinary vaccines to control bacterial infections** **(Gary Entrican)**
13h00				**12h30:** Introduction and Aims **12h40:** Importance of designing the vaccine TPP for translational research *(Paul Wood, Monash University, Australia)* **12h50:** Outer membrane vesicles (OMVs) for bacterial vaccinology *(Adam Cunningham, BactiVac, UK)* **13h00:** Bacterial glycoconjugate vaccines (delivered by the panel) *(Brendan Wren, LSHTM, UK) (pre-recorded)* **13h10:** Viral vectors for veterinary vaccinology *(Michael Jarvis, The Vaccine Group, UK) (pre-recorded)* **13h20:** mRNA platforms for veterinary vaccinology *(Helba Bredell, Afrigen, SA)* **13h30:** STAR-IDAZ vaccine roadmaps *(Gary Entrican, University of Edinburgh, UK)* **13h40:** DISCONTOOLS bacterial vaccines gaps *(Johannes Charlier, Kreavet, Belgium)*
14h00	Registration for IVIS 2023 Opens & Poster Setup Commences	Not in use	13h50	Initial Q&A, format of breakout groups, Assembly and Discussions
			14h10	Coffee/Networking/Opportunity for Initial Informal Feedback
			14h30	Breakout Discussions

VENUE	VENUE 1 – NDLOPFU	VENUE 2 - INGWE/MHELEMBE		VENUE 3 - NDAU/NARI
			15h10	Rapporteur Summaries
			15h40	Panel Discussion with speakers and chairs
			16h10	Capture the agreed workshop outcomes
			16h30	Final comments and close
17h00	**Welcome and Opening** *(Prof. Sven Parsons, Afrivet & Dr Claire Rogel-Gaillard, INRAE)*			
17h15	**Keynote: Avian Influenza: An African perspective** *(Prof. Celia Abolnik, University of Pretoria)*			
18h15	**Keynote: What Innovative approaches and technologies would be applicable for vaccine development and production in Africa?** *(Prof. Baptiste Dungu, Design Biologix and University of Kinshasa)*			
19h30	Welcome Reception at the Safari Lodge Hotel (dress code, smart casual)			

Day 2 - Saturday 18 November

07h30 – 08h30	Registration and Arrival Coffee/Tea					
VENUE	VENUE 1 – NDLOPFU		VENUE 2 - INGWE/MHELEMBE		VENUE 3 - NDAU/NARI	
08h30	Session 1.1.1		Session 1.2.1		Session 1.3.1	
	INNATE IMMUNITY *Chairs: Teresa Freire & Yolandy Lemmer*		IMMUNO-INFORMATICS *Chairs: Christine Maritz-Olivier & Michelle Baker*		IMMUNOLOGY OF VIRAL DISEASES (1) *Chairs: Celia Abolnik & Gerald Chege*	
	Presenter	Abstract nr. and Title	Presenter	Abstract nr. and Title	Presenter	Abstract nr. and Title
08h30	Werling, Dirk	The role of bovine dectin-1 in inducing trained immunity and cross-protection	Malgwi, Samson	(025) Reverse vaccinology approach in designing a multiepitope-based vaccine against *Babesia* from rhoptry-associated protein 1 (RAP-1) antigen	Barone, Lucas	(225) Bovine viral diarrhoea virus (BVDV) preferentially infects arginase-producing bovine monocyte-derived macrophages
08h48			Wattrang, Eva	(058) Single-cell RNA-seq mapping of chicken peripheral blood leukocytes	Nedumpun, Teerawut	(057) Cyclooxygenase (COX)-2 inhibitor inhibited porcine reproductive and respiratory syndrome virus (PRRSV) type 2-induced M2 macrophage polarization
09h06	Bettin, Leonie	(068) Contributions of porcine gamma-delta T cells to antiviral immunity: An investigation into Toll-like receptor expression and potential cytotoxic activity	Adeleke, Matthew A.	(162) Immuno-informatics analysis of antigens as candidate vaccines against Eimeria and Toxoplasma for chickens	Summerfield, Artur	(072) A systems immunology approach to identify protective and detrimental innate and adaptive immune responses following ASFV infection of pigs

VENUE	VENUE 1 – NDLOPFU		VENUE 2 - INGWE/MHELEMBE		VENUE 3 - NDAU/NARI	
	Session 1.1.1		**Session 1.2.1**		**Session 1.3.1**	
09h24	Faber, Erika	(116) Virulent African horse sickness virus serotype 4 interferes with the innate immune response in horse peripheral blood mononuclear cells *in vitro*	Not in use		Kaeser, Tobias	(107) Modified-live virus vaccination induces heterologous immunity against different type-2 PRRSV strains
09h42	Not in use		Not in use		Di Placido, Marie	(146) Characterization of foot-and-mouth disease virus cross-serotypes reactive and neutralizing antibodies
10h00	Refreshment Break in Foyer					
10h30	**Session 1.1.2**		**Session 1.2.2**		**Session 1.3.2**	
	TISSUE-SPECIFIC IMMUNITY *Chair: Sven Parsons*		**ZOONOSES** *Chair: Gilles Foucras & Michelle Miller*		**IMMUNOLOGY OF BACTERIAL DISEASES** *Chair: Femke Broere & Wynand Goosen*	
10h30	Chapuis, Ambre *(presented by Hope, Jayne)*	(029) Characterization of bovine and ovine ileal organoids after polarity exchange.	Dorhoi, Anca	(041) Host adaptation of Lagos bat lyssavirus is driven by inhibition of type I interferon	Bresser, Laura	(030) Chitosan hydrogel induces immune cell recruitment in the bovine mammary gland and increases cure rates of intramammary infections caused by *Staphylococcus* spp.
10h48	Jungersen, Gregers	(050) Induction of antigen-specific secretory IgA in small intestine of newborn piglets after parenteral immunization with retinoic acid-enhanced adjuvant	Freire, Teresa	(015) *Fasciola hepatica* infection in cattle alters the immunity induced by respiratory vaccine	Berghaus, Londa	(156) The impact of age on vitamin D receptor expression, vitamin D metabolism and associated cytokines in ex vivo *Rhodococcus equi* infection of equine alveolar macrophages
11h06	Myslinska, Joanna	(088) Precision-cut lung slices (PCLS) as a platform to study *Mycoplasma hyopneumoniae* interactions with porcine lung tissue	Marti, Andrea	(108) Adaptation of Japanese encephalitis virus in pigs can enhance virulence, virus replication, viral shedding and innate immune responses	Dorhoi, Anca	(046) Modelling immune responses of cattle to mycobacteria using magnetic bioprinted granulomas
11h24	Crisci, Elisa	(128) The role of pathogens and anti-PRRSV immunity in the Porcine Respiratory Disease Complex	**IMMUNOLOGY OF WILDLIFE AND EXOTICS**		Not in use	
			Seguel, Mauricio	(081) Maternal care favours development of distinct immunotypes in a marine mammal		
11h42	Not in use		Steinbach, Falko	(176) Elephant Endotheliotropic Herpesvirus (EEHV) Vaccine and First in-Elephant, Proof-of-Concept Trial	Not in use	
12h00	POSTER SESSION 1					
13h30	Lunch in the Safari Lodge					

VENUE	VENUE 1 – NDLOPFU		VENUE 2 - INGWE/MHELEMBE		VENUE 3 - NDAU/NARI	
14h30	Session 1.1.3		Session 1.2.3		Session 1.3.3	
	ADAPTIVE IMMUNITY *Chair: Alejandra Capozzo & Francois Maree*		IMMUNOLOGY OF PARASITE DISEASES *Chair: Eva Wattrang & Christian Stutzer*		VACCINE DEVELOPMENT (1) *Chair: Vish Nene & Marietha O'Kennedy*	
14h30	Okagawa, Tomohiro	(019) PD-L1 blockade enhances T-cell response to vaccination in calves	Hiromi Okino, Cintia	(002) Improved mucosal response against hemonchosis related to βA allele of ovine beta-globin gene	Lemmer, Yolandy	(036) Immunogenicity of a plant-produced African horse sickness polyvalent vaccine validated in IFNAR mice.
14h48	Crowley, Dan	(023) Diet and defences: low affinity antibodies in bats are affected by food quality	Da Silva Costa, Monique	(007) Effects of experimental *Fasciola hepatica* infection on the long-term immune response of the foot and mouth disease vaccine	Hope, Jayne	(042) Single-cell RNA-Seq Reveals Multiple Sub-Populations of Bovine Afferent Lymphatic Dendritic Cells Draining the Skin
15h05	Refreshment Break					
15h45	Takeuchi, Hiroto	(063) Exploring the therapeutic potential of canine TGF-β decoy receptor for melanoma that reverses TGF-β1–mediated immunosuppression	Wattrang, Eva	(049) Local immune responses to *Eimeria tenella* infection in immune chickens – a possible role for interferon-γ induced genes in the inhibition of parasite replication	Ashley, Emily	(043) Evaluation of a Live Attenuated Pseudorabies Virus-Vectored Nipah Virus Vaccine
16h03	Lauterkorn, Nira & Deosthali, Samruddhi	(087) Transwell cultures of lymph node derived cells for the *in vitro* monitoring of adaptive immune responses in pigs	Oser, Larissa	(077) Dynamics of mucosal and systemic Th2 / Th1 effector T cells in response to tissue migrating *Ascaris suum* infections	Jungersen, Gregers	(051) Vaccine immunogenicity in pigs after immunization at different injection sites with liposome-based adjuvants containing specific immunomodulators
16h21	Not in use		Hellman, Stina	(157) Equine organoid-derived monolayers as a novel *in vitro* model to study host-parasite interactions	Not in use	
16h38	Close of Day2					
18h30	Boma Braai at Cattle Baron *(only for guests who pre-registered)*					

Day 3 – Sunday 19 November

07h30 – 08h30	Registration and Arrival Coffee/Tea			
VENUE	VENUE 1 – NDLOPFU		VENUE 2 - INGWE/MHELEMBE	
08h30	Session 2.1.1		Session 2.2.1	
	WORKSHOP 3 - IMMUNOLOGICAL TOOLKIT SESSION *Chair: Jayne Hope*		IDRC PANEL SESSION - From Bench to Market: Livestock Vaccine Development in and for low- and middle-income countries	
	Presenter	Abstract nr. and Title	**08h30:** Welcome *(Dr Musa Mulongo)* **08h35:** Opening *(Dr Andy Peters)* **08h55:** Panel Discussion 1 - Multivalent approach for the improvement of inactivated vaccines against small ruminant diseases in Africa *(Dr Hezron Wesonga, KALRO)* **09h05**: Panel Discussion 2 - Clinical trials to develop a subunit vaccine for contagious bovine pleuropneumonia in Kenya *(Dr Elise Schieck, ILRI)* **09h15**: Q&A Session **09h20:** Panel Discussion 3 - Improvement of Theileria parva sporozoite vaccine (ITM) against East Coast Fever *(Maxime Madder, ClinGlobal)* **09h30:** Panel Discussion 4 - Development of 2 multivalent RVF vaccines for improved uptake in cattle and in small ruminants *(Dr Lamia Rafi, MCI)* **09h40:** Public Q&A Session	
08h30	Hamid, Benjamin-Layla	(071) CD38 expression on porcine αβ-T-cell subsets and its role in T-cell activation		
08h40	Dry, Inga	(037) The Immunological Toolbox: Advancing veterinary immunology research through the generation of novel reagents		
08h50	Di Placido, Marie	(147) A customizable suite of methods to sequence and annotate cattle antibodies		
09h00	Entrican, Gary	Recombinant antibodies driving immunological research		
09h10	Hammond, John	The future of the Toolbox		
09h20	Round Table facilitated discussions			
09h50	Feedback from discussions via rapporteurs			
10h00	Refreshment Break			
10h30	Session 2.1.2		Session 2.2.2	
	WORKSHOP 4 - VIC MHC *Chair: John Hammond*		IMMUNODIAGNOSTICS *Chair: Yolandy Lemmer & Joan Lunney*	
	Presenter	Abstract nr. and Title	Presenter	Abstract nr. and Title
10h30	Hammer, Sabine E.	(020) Comparative analysis of swine leukocyte antigen (SLA) gene diversity in Göttingen Minipigs	Khalid, Hamza *(presented by Hope, Jayne)*	(012) Investigating the biomarker potential and development of lateral flow assays to detect host proteins for improved diagnosis of bovine tuberculosis
10h48	Robert, Jacques	(067) Role of nonpolymorphic MHC-I and innate-like T cells in resistance and tolerigenic neonatal immunity to mycobacteria	Sengun, Ezgi	(005) Revealing the sheep immune cell composition using multidimensional flow cytometry
11h06	Rogel-Gaillard, Claire	(129) A revised view of putative functional histocompatibility genes in the SLA complex	Fehrsen, Jeannie *(presented by Van Schalkwyk, Antoinette)*	(161) Identification of novel epitopes capable of differentiating between vaccine and field lumpy skin disease virus.
11h24	Schwartz, John C.	(126) Haplotypic and allelic diversity of non-classical MHC class I in ruminants	Not in use	
11h42	Hammond, John	An update on species and tools on IPD-MHC	Not in use	
12h00	POSTER SESSION 2			
13h00	Lunch			

VENUE	VENUE 1 – NDLOPFU
14h00	GOLD SPONSOR PRESENTATION: CSIRO
14h10	**Plenary 1: Local adaptive immunity in pigs – getting the right balance?** *(Dr Wilhelm Gerner, The Pirbright Institute)* *Chair: Eva Wattrang*
15h10	**Distinguished Veterinary Immunologist - Award & Presentation** *(Dr Joan Lunney)* *Chairs: Claire Rogel-Gaillard & Paul Wood*
16h10	Close of Day 3
16h30	Game Drive (limited to pre-registered guests)
19h00	Official Symposium Dinner under the African Sky *(dress code casual and something warm for the cool evening)**

Day 4 – Monday 20 November

07h30 – 08h30	Registration and Arrival Coffee/Tea			
Venue	VENUE 1 – NDLOPFU		VENUE 2 - INGWE/MHELEMBE	
08h30	Session 3.1.1		Session 3.2.1	
	IMMUNOGENOMICS & RESISTANCE TO DISEASE *Chair: Claire Rogel-Gaillard & Ivy Tshilwane*		VACCINE DEVELOPMENT (2) *Chair: Baptiste Dungu & Melany Chitray*	
	Presenter	Abstract nr. and Title	Presenter	Abstract nr. and Title
08h30	Foucras, Gilles	(132) Inborn errors of immunity in domestic ruminants: lessons learned from two examples	Win, Shwe Yee	(065) In vitro evaluation of cysteine protease and ferritin 2 as vaccine antigens with broad efficacy across avian mites, poultry red mites, tropical fowl mites, and northern fowl mites
08h48	Kammerer, Robert	(160) Living with only two arms: The TRDC-knockout pigs lacking γδ T cells	Devriendt, Bert	(091) Aminopeptidase N-mediated transport across the small intestinal epithelium drives gut immunity to oral vaccine antigens in pigs
	IMMUNE-REGULATION AND MODULATION			
09h06	Riva, Federica	(079) Bovine colostrum supplementation in rabbit diet modulates gene expression of cytokines, gut vascular barrier and red-ox related molecules in the gut wall	Gourapura, Renukaradhya	(120) Mannose-chitosan nanoparticle surface adsorbed inactivated influenza virus vaccine elicits cross-protective humoral immunity in pigs
09h24	Not in use		Mashudu, Nesane	(221) Single dose new generation heartwater vaccine for smallholder farmers
09h42	Not in use		Steinbach, Falko	(149) The ISG15 network is crucial and tightly regulated in the early protection of classical swine fever virus C strain vaccine
10h00	Refreshment Break			

Venue	VENUE 1 – NDLOPFU		VENUE 2 - INGWE/MHELEMBE	
10h30	**Session 3.1.2**		**Session 3.2.2**	
	COMPARATIVE IMMUNOLOGY *Chair: Gregers Jungersen & Chris Marufu*		**IMMUNOLOGY OF VIRAL DISEASES (2)** *Chair: Alri Pretorius & Artur Summerfield*	
10h30	Le Nours, Jerome	(013) Molecular insights into the family of MHC-like molecules (UTs) from marsupials	Capozzo, Alejandra	(010) Avidity ELISA as an alternative to the Virus Neutralization test to assess Foot-And-Mouth Disease vaccine-induced antibody responses in buffaloes (Bubalus Bubalis)
10h48	Görig, Samira Christin	(032) Pathogen recognition by bovine myeloid C-type lectin receptors	Silva, Ediane	(190) African Swine Fever: Cellular and Humoral Immune Response of pigs vaccinated with Live Attenuated Strain
11h06	Miller, Laura	(085) How do *Odocoileus virginianus* (white-tailed deer) weather SARS-CoV-2 infection?	Rojas, José M	(208) Peste des petits ruminant's virus activates goat dendritic cells but impairs antigen presentation
11h24	Devriendt, Bert	(086) Transcriptional profiling of blood and liver Natural Killer cells reveals the presence of a novel liver Natural Killer cell subset in swine.	Sevilla, Noemi	(209) Comprehensive immune profiling reveals that arbovirus infection activates immune checkpoints during acute t cell immunosuppression
11h42	Noble, Alastair *(presented by Graham, Simon)*	(090) Distinct effector functions mediated by Fc regions of bovine IgG subclasses and their interaction with Fc gamma receptors	Louloudes-Lazaro, Andres	(222) Bluetongue virus disrupts the type-I interferon response by interfering with the cGAS pathway
12h00	POSTER SESSION 3			
13h00	Lunch			
14h00	**Plenary 2: Immunogenomics and resistance to disease: antiviral immunity in bats** *(Dr Michelle Baker, CSIRO)* *Chairs: Alejandra Capozzo*			
15h00	**Keynote: *Streptococcus* suis interactions with B lymphocytes: uncovering the role of IgM** *(Prof. Mariela Segura, Université de Montréal)*			
16h00	Body break (10 mins)			
16h10	**Keynote: Veterinary Immunology: from knowledge to solutions** *(Dr Samuel Thevasagayam, Bill and Melinda Gates Foundation)* *Chair: Vish Nene*			
17h00	Close of Symposium, Poster Awards and hand-over to 2025 IVIS hosts			

Day 5 – Tuesday 21 November

VENUE	VENUE 1 – NDAU/NARI	VENUE 2 - INGWE/MHELEMBE
08h30 – 10h00	Not in use	LVIF Final Event Round table dissection (closed event)
10h00 – 11h00	Light brunch in Conference Foyer	Refreshment Break (LVIF)
11h00 – 13h00	**Workshop 5** Supporting early career researchers in veterinary immunology and vaccinology research *(International Veterinary Vaccinology Network (IVVN))*	LVIF Final Event (closed event)
12h30 – 13h15	Close of Symposium	Lunch (LVIF)
13h15 – 15h00	Not in use	LVIF Final Event (closed event)
15h00 – 15h30		Refreshment Break (LVIF)
15h30 – 17h00		LVIF Final Event (closed event)

Day 2 – Saturday 18 November

Poster Session	Theme	Presenter	Title
1 – 12h15	Innate Immunity	Riva, Federica	(080) Different immune response against Gram positives and Gram negative mammary infection revealed by two dairy cow breeds.
1 – 12h15	Innate Immunity	Broere, Femke	(082) Characterization of polarization states of canine monocyte derived macrophages
1 – 12h15	Innate Immunity	Rodriguez, Natalia	(113) Immunological Response to Increased Intestinal permeability on Endurance Exercise Horses
1 – 12h15	Innate Immunity	Matis, Gabor	(123) The immunomodulatory action of the antimicrobial peptide IDR-1002 in a hepatic cell culture model of chicken origin
1 – 12h15	Innate Immunity	Foucras, Gilles	(133) Which factors modulate the bovine whole blood response to LPS: Nature vs Nurture?
1 – 12h15	Innate Immunity	Ardali, Razieh	(150) In vitro screening of immunostimulatory ligands for induction of trained immunity in porcine monocyte/macrophages
1 – 12h15	Innate Immunity	Ardali, Razieh	(152) Harnessing trained immunity to enhance resistance of piglets against infections
1 – 12h15	Innate Immunity	Riva, Federica	(177) Canine amniotic fluid at birth: From a discarded sample to a potential diagnostic of neonatal maturity and health.
1 – 12h15	Innate Immunity	Franzoni, Giulia	(187) Heterogeneity of Phenotypic and Functional Changes to Porcine Monocyte-Derived Macrophages Triggered by Diverse Polarizing Factors In Vitro
1 – 12h15	Innate Immunity	Castillo, Amanda	(189) PMN support *Brucella* dispersal with reduced immune recognition
1 – 12h15	Adaptive Immunity	Brunner, Milena	(022) Unravelling signals important for early chicken B-cell development
1 – 12h15	Adaptive Immunity	Maekawa, Naoya	(075) Immune checkpoint blockade for cancer immunotherapy in dogs: updated results from a clinical study using anti-PD-L1 antibody (c4G12)
1 – 12h15	Adaptive Immunity	Broere, Femke	(083) Cellular and Humoral immune responsiveness to inactivated Leptospira interrogans in dogs vaccinated with a tetravalent Leptospira vaccine

Poster Session	Theme	Presenter	Title
1 – 12h15	Adaptive Immunity	Fanning, Liam	(111) Immune biome of Bovine Colostrum
1 – 12h15	Adaptive Immunity	Schwartz, John C	(144) The influence of the germline antibody loci on the pig and warthog antibody repertoire
1 – 12h15	Adaptive Immunity	Perez-Filgueira, Mariano	(154) Increased antigenic broadness in the adaptive humoral immunity elicited by different Foot and Mouth Disease vaccine regimes against heterologous viral strains.
1 – 12h15	Adaptive Immunity	Mair, Kerstin	(159) Characterization of novel memory marker on porcine T cells
1 – 12h15	Adaptive Immunity	Woziri, Abubakar	(185) Evaluation of haematological biomarkers of immune response in chickens following *in vivo* administration of inactivated Avian Influenza H5 vaccine
1 – 12h15	Immunology of viral diseases 1	Hiromi Okino, Cintia	(016) Essential oils able to completely inhibit the avian coronavirus replication.
1 – 12h15	Immunology of viral diseases 1	Nedumpun, Teerawut	(056) Different macrophage polarization patterns induced by porcine reproductive and respiratory syndrome viruses (PRRSV)
1 – 12h15	Immunology of viral diseases 1	Crisci, Elisa	(110) Predict and Protect against PRRSV (PreProPRRSV): Combining PRRSV forecasting technology with vaccine efficacy prediction to prevent PRRSV outbreak.
1 – 12h15	Immunology of bacterial diseases	Gottschalk, Marcelo	(098) IgM antibodies play a major role in the elimination of *Streptococcus suis* serotype 2
1 – 12h15	Immunology of bacterial diseases	Gottschalk, Marcelo	(099) *Streptococcus suis* surface-antigen recognition by antibodies and bacterial elimination is influenced by capsular polysaccharide structure
1 – 12h15	Immunology of parasite diseases	Hiromi Okino, Cintia	(028) Ovine resistance against *Haemonchus contortus*: Does a breed or a β-globin subtype feature?
1 – 12h15	Immunology of parasite diseases	Ndaba, Bongeka	(191) Evaluation of global immune responses in goats infected with *Haemonchus contortus* using RNA sequencing
1 – 12h15	Immunology of parasite diseases	Stutzer, Christian	(227) A Comparative immunological profile of *Babesia microti* infected BALB/c mice co-infested with *Ixodes ricinus* ticks and its effect on immunisation
1 – 12h15	Immunology of parasite diseases	Stutzer, Christian	(228) *In vivo* evaluation of *Ixodes ricinus* induced effects on T and B-cell maturation in the spleen and lymph nodes of BALB/c mice
1 – 12h15	Vaccine Development 1	Chiweshe, Stephen	(014) Comparison of immunogenicity of vaccine payloads delivered by 3 different viral vaccine vectors.
1 – 12h15	Vaccine Development 1	Beddoe, Travis	(039) The development of a subunit vaccine for the control of crocodilepox virus infection in farmed Australian saltwater crocodiles (*crocodylus porosus*)
1 – 12h15	Immunoinformatics	Gambu, Asanda	(026) *In silico* prediction of CD4+ T cell and B cell epitopes targeting *H. contortus* vaccine antigens
1 – 12h15	Immunoinformatics	van der Byl, Cara	(114) In silico prediction of CD4 T cell and B cell epitopes of the peptidase and GTPase protein families of *Haemonchus contortus*
1 – 12h15	Immunoinformatics	Bambeni, Thandikhaya	(186) *In silico* analysis of GPI-anchored hypothetical proteins from Babesia bovis as potential vaccine candidates
1 – 12h15	Zoonoses	Ghielmetti, Giovanni	(139) *Mycobacterium tuberculosis* in a captive African Elephant: identification of mixed infection Using Whole Genome Sequence Data
1 – 12h15	Zoonoses	Martins de Camargo, Maristela	(164) Saliva as a non-invasive tool for monitoring microbial diversity and pathogens in wildlife
1 – 12h15	Zoonoses	Chege, Gerald	(168) The use of interferon-gamma releasing assays (IGRA) to improve the detection of tuberculosis in captive-bred non-human primates

Poster Session	Theme	Presenter	Title
1 – 12h15	Zoonoses	Nelson, Bukamba	(195) Isolation of bacteriophages against salmonella isolates from environmental water samples and gorilla feces collected from Bwindi impenetrable national park
1 – 12h15	Immunology of wildlife and exotics	Gumbo, Rachiel	(035) Cytokine release assay for the detection of Mycobacterium bovis infection in African Lions (Panthera leo), Cheetahs (Acinonyx jubatus) and Leopards (Panthera pardus)
1 – 12h15	Immunology of wildlife and exotics	Espejo, Camila	(158) Dexamethasone increases in vitro immune cell proliferation in response to M. bovis in African buffalo.
1 – 12h15	Immunology of wildlife and exotics	Sarker, Palash	(184) Strengths and Opportunities of One Health Approach for Rabies Control in the Jhalakhati District, Bangladesh, 2022
1 – 12h15	Immunology of wildlife and exotics	Kayigwe, Ahab	(213) Proteogenomic analysis reveals lncRNA-encoded immunopeptides in the devil facial tumour disease
1 – 12h15	Immunology of wildlife and exotics	Roberts, Laura C.	(226) Vaccination of African Penguins (*Spheniscus demersus*) against H5 high pathogenicity avian influenza: a comparison between an inactivated whole-virus vaccine and a plant-produced virus-like particle

Day 3 – Sunday 19 November

Poster Session	Theme	Presenter	Title
2 – 12h00	Immunodiagnostics	Hammer, Sabine E.	(034) Revisiting the correlation of *c-Kit* mutation status and treatment decisions in canine mast cell tumours
2 – 12h00	Immunodiagnostics	Chase, Chris	(134) Leukocyte dynamics in vaccinated and unvaccinated animals following dual challenge with Bovine Viral Diarrhea Virus-Mannheimia haemolytica model to compare vaccine efficacy in calves.
2 – 12h00	Immunodiagnostics	Dlamkile, Zinathi	(155) Development of a reverse transcription quantitative real-time PCR assay for the evaluation of a multivalent inactivated vaccine
2 – 12h00	Immunodiagnostics	Capozzo, Alejandra	(172) Assessment of serum IgG-avidity and IgG1 titres as predictors of heterologous protection against foot-and-mouth disease in vaccinated cattle
2 – 12h00	Immunodiagnostics	Marufu, Chris	(175) Comparative analysis of four diagnostic methods to detect *Fasciola* species in naturally infected slaughter dairy cattle
2 – 12h00	Immunodiagnostics	van der Heijden, Elise	(193) The MicroRNAome of Cattle Infected with Mycobacterium bovis: Towards the Characterization of Potential Novel Biomarkers
2 – 12h00	Immunodiagnostics	Moabelo, Khomotso	(076) Comparison of the Bionote NSP Ab ELISA and PrioCheck FMDV NS ELISA
2 – 12h00	Toolkit	Dry, Inga	(017) Fc-tagged fusion proteins as tools to define veterinary cytokines and investigate immunological receptor/ligand interactions
2 – 12h00	Toolkit	Gourapura, Renukaradhya	(101) Generation and characterization of swine immune reagents for monitoring pig immune status and for biomedical research
2 – 12h00	Toolkit	Schmidt, Selma	(136) CD25, CD40L and CD69 as activation induced markers (AIMs) in porcine T cells
2 – 12h00	Toolkit	Di Placido, Marie	(148) Comparing flow cytometry and bulk transcriptomics with single cell gene expression to characterize cattle B cell subsets
2 – 12h00	Teaching immunology	Oumouna, Mustapha	(214) African Vaccinology Network (AfVANET): an African network by African scientists
2 – 12h00	Tissue-specific immunity	Mackei, Máté	(151) Effects of chicoric acid on a primary hepatic co-culture of chicken origin treated with viral RNA analogue poly I:C

Day 4 – Monday 20 November

Poster Session	Theme	Presenter	Title
3 – 12h00	Immunology of viral diseases 2	Nefefe, Tshifhiwa	(145) Host immune response to FMDV SAT 1, 2 and 3 infections: Defining mechanisms of persistent infection in cattle.
3 – 12h00	Immunology of viral diseases 2	Mtimunye, Mpendulo	(188) Host immune response to FMDV SAT 1, 2 and 3 infections: Defining mechanisms of persistent infection in cattle.
3 – 12h00	Immunology of viral diseases 2	Yiltawe, Wungak	(212) Foot and Mouth Disease Vaccine Immunogenicity Study Among the Agro-Pastoralists In Plateau State, Nigeria
3 – 12h00	Vaccine Development 2	Wattegedera, Sean	(040) Induction of humoral and cellular responses to parapox virus-vectored antigen for vaccine development
3 – 12h00	Vaccine Development 2	Gottschalk, Marcelo	(047) Immune response against bacterins used to control *Streptococcus suis* infections in pigs
3 – 12h00	Vaccine Development 2	Gopakumar, Gayathri	(048) Clinical assessment and transcriptome analysis of host immune responses in a vaccination-challenge study using a glycoprotein G deletion mutant vaccine strain of infectious laryngotracheitis virus
3 – 12h00	Vaccine Development 2	Lacasta, Anna	(053) New advances in the development of a subunit vaccine targeting antibody production against African swine fever.
3 – 12h00	Vaccine Development 2	Oboge, Harriet	(055) Spontaneous Nanoliposome Antigen Particleization (SNAP) - a Novel Subunit Vaccine Technology for Livestock Vaccine Development
3 – 12h00	Vaccine Development 2	Corripio-Miyar, Yolanda	(059) Development of a novel recombinant subunit Q fever vaccine for ruminants
3 – 12h00	Vaccine Development 2	Cottingham, Ellen	(060) Virally-Vectored Immunocontraceptives for the Management of Feral Cats in Australia
3 – 12h00	Vaccine Development 2	Kolakowski, Jeannine	(070) Evidence of glycosylation in the schizont life cycle stage of Theileria parva parasites
3 – 12h00	Vaccine Development 2	Hiromi Okino, Cintia	(074) Transcriptomic profiling shows the induction of humoral and cellular response-related genes in pigs following vaccination with an Influenza A nanovaccine
3 – 12h00	Vaccine Development 2	Chege, Hannah	(078) Identification of new antigens as potential sub-unit vaccine candidates for ECF control
3 – 12h00	Vaccine Development 2	Gottschalk, Marcelo	(097) Influence of maternal antibodies on the immune response of young piglets vaccinated with a *Streptococcus suis* serotype 2 bacterin
3 – 12h00	Vaccine Development 2	Li, Hao	(100) Immunogenicity and Protective Efficacy of Ag85A and truncation of PstS1 fusionprotein vaccines against tuberculosis
3 – 12h00	Vaccine Development 2	Borrego, Belen	(102) Safety and efficacy of the Rift valley fever live-attenuated vaccine candidate 40Fp8 in pregnant ewes
3 – 12h00	Vaccine Development 2	Gourapura, Renukaradhya	(103) Characterization of the efficacy of a split swine influenza A virus nasal vaccine formulated with a nanoparticle/STING agonist combination adjuvant in conventional pigs
3 – 12h00	Vaccine Development 2	Hayes, Jack	(104) Isolation of porcine reproductive and respiratory syndrome virus glycoprotein specific monoclonal antibodies from hyperimmune pigs
3 – 12h00	Vaccine Development 2	Chitray, Melanie	(105) Foot-and-mouth disease SAT specific virus peptide phage display libraries for the identification of epitopes
3 – 12h00	Vaccine Development 2	Crisci, Elisa	(109) Intranasal Ad5 influenza vaccine elicits hemagglutinin-specific antibody response in pregnant and lactating pigs.
3 – 12h00	Vaccine Development 2	Kaeser, Tobias	(117) The pig as a biomedical animal model to develop treatment and prevention strategies against *Chlamydia trachomatis*
3 – 12h00	Vaccine Development 2	Cox, Eric	(118) Human whole-cell / B-subunit oral cholera vaccines Duochol and Dukoral induce antibody responses in a porcine model.
3 – 12h00	Vaccine Development 2	Chase, Chris	(121) Protection of neonatal, colostrum-fed calves with a modified live, intranasal, tri-valent vaccine using an experimental challenge with virulent bovine respiratory syncytial virus

Poster Session	Theme	Presenter	Title
3 – 12h00	Vaccine Development 2	Fortes de Brito, Rory	(122) Assessment of immunogenicity and efficacy of an attenuated herpesvirus-based vector expressing highly conserved porcine reproductive and respiratory syndrome virus M and NSP5 proteins
3 – 12h00	Vaccine Development 2	Chitray, Melanie	(127) Construction of a FMDV-specific recombinant antibody phage-display bovine libraryfor epitope identification and diagnostic reagents
3 – 12h00	Vaccine Development 2	Edwards, Jane	(137) Development of methods to isolate natural porcine antibodies that broadly neutralise porcine reproductive and respiratory syndrome viruses
3 – 12h00	Vaccine Development 2	Chabalgoity, Jose A.	(140) A transcriptional signature of effective vaccination in bovines to Foot-and-Mouth Virus Disease (FMDV)
3 – 12h00	Vaccine Development 2	Fisch, Andressa	(142) Applying phage display screening and next-generation sequencing to identify B-cell epitopes from tick salivary antigens
3 – 12h00	Vaccine Development 2	Young, Alan	(165) A USDA-licensed platform approach for the rapid generation and commercial deployment of bacterial subunit vaccines against emerging diseases.
3 – 12h00	Vaccine Development 2	Maichomo, Monicah	(201) Early stages of developing a subunit bacterial vaccine against camel mastitis
3 – 12h00	Vaccine Development 2	O'Kennedy, Martha	(215) Assembling Newcastle disease virus-like particles in Nicotiana benthamiana plants as potential vaccine
3 – 12h00	Vaccine Development 2	O'Kennedy, Martha	(216) Efficacy of a plant-produced infectious bronchitis virus-like particle vaccine in specific pathogen-free chickens
3 – 12h00	Vaccine Development 2	Mulongo, Moses	(217) TAHSSL: A new R&D and Commercialization Platform at ILRI
3 – 12h00	Vaccine Development 2	Masembe, Charles	(219) No longer a regional problem: molecular evolution and transmission of African swine fever on the African continent
3 – 12h00	Vaccine Development 2	Wilda, Maximiliano	(223) Polysaccharide microparticles as carriers of a recombinant antigen against GnRH in oral vaccination in male rats.
3 – 12h00	Vaccine Development 2	Miller, Laura	(224) Validation of a Modified Live Virus (MLV) prototype of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) with replication-competent expression of porcine interferons (IFNs)
3 – 12h00	Vaccine Development 2	El Wahli, younès	(229) Protective Efficacy Evaluation of Four Inactivated Commercial Vaccines Against Low Pathogenic Avian Influenza H9N2 Virus Under Experimental Conditions in Broiler Chickens
3 – 12h00	Immune-regulation and modulation	Matthews, Megan Ceris	(069) Investigating the soil mycobacteriome to validate M. bovis tuberculin skin test results
3 – 12h00	Comparative Immunology	Divín, Daniel	(004) Cannabinoid receptor 2 evolutionary gene loss makes parrots more susceptible to neuroinflammation
3 – 12h00	Comparative Immunology	Hammer, Sabine E.	(033) Exploratory screening for miRNA biomarkers in canine multicentric lymphoma
3 – 12h00	Comparative Immunology	Hart, Kelsey	(166) Effects of hydrocortisone, ascorbic acid, and thiamine treatment on immune responses in healthy neonatal foals
3 – 12h00	Comparative Immunology	Mansilla, Florencia	(170) MDP-mediated chronic NOD2 stimulation confers protection against LPS challenge through M2b macrophage polarization in mice
3 – 12h00	Immunogenomics & resistance to disease	Morrison, Liam	(131) Continent-wide population genomics of the African buffalo (*Syncerus caffer*) indicates infectious disease as a significant selective pressure

Keynote Presentations

Avian influenza- an African perspective

Celia Abolnik, MSc, PhD

Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria - celia.abolnik@up.ac.za

Avian influenza is a serious disease on multiple fronts: devastating outbreaks in poultry threaten global food production, outbreaks in endangered wild birds threaten biodiversity, and emerging strains with the ability to infect mammals spark fears that it may cause the next human pandemic. Historically, high pathogenicity avian influenza (HPAI) viruses occasionally emerged in poultry via mutation but remained localized, and outbreaks could be stamped out through mass culling. However, the Goose/Guangdong (Gs/GD) H5 HPAI viral lineage, that first emerged in China in the mid 2000's, gained the ability to infect some waterfowl without killing them, and began spreading in migratory birds. Now, a reservoir of infection is established in the northern hemisphere, from which Gs/GD H5 HPAI viruses are disseminated by migrating ducks. This means that HPAI viruses are being re-introduced to countries on an almost annual basis to cause new epidemics in their poultry production systems, and the traditional method of control by stamping out has become ineffective and unstainable. Decades of research into the ecology of avian influenza is centered on temperate northern hemisphere conditions, where bird breeding and migration routes are well defined and regulated by seasonal temperatures, and the abundance of water, but there are different ecological (and economic) factors at play in the tropical and sub-tropical global south that affect the detection and control of avian influenza. For example, much less predictable wild bird movements and asynchronous breeding patterns that are more dependent on rainfall patterns than temperature, higher UV exposure, and relatively less and warmer surface water. Furthermore, South Africa is the largest global export producer of a unique poultry specie that is susceptible to infection with influenza viruses, namely ostriches. In this context, the unique challenges faced by South Africa in recent H5 and H7 HPAI outbreaks and options for this disease's control will be discussed.

Biography: Prof. Abolnik joined the Faculty of Veterinary Science in August 2012 as the South African Poultry Association's Research Chair in Poultry Health and Production at the level of associate professor. In January 2018 she was appointed to the NRF-DST South African Research Chair (SARChI) in Poultry Health and Production, the Faculty's first and only SARChI, and was promoted to a tenured full professor. One postdoctoral fellow, four PhD and eight MSc students obtained their degrees under her supervision and her current research group comprises two postdoctoral fellows, two PhD students, two MSc students and a junior technical assistant. Past and present key collaborations include APHA (UK), SEPRL (USA), IZSVe (Italy), James Cook University (Australia), CIRAD (France), CVL (Nigeria) and locally the CSIR, University of Stellenbosch, national veterinary laboratories and poultry industry and government stakeholders. She is currently a C2-rated NRF scientist with 68 peer-reviewed scientific papers.

What Innovative approaches and technologies would be applicable for vaccine development and production in Africa?

Baptiste Dungu, DVM MSc PhD

Design Biologix and University of Kinshasa - badungu@gmail.com

Africa's livestock population has been steadily increasing, which is considered to be a positive trend, given the increasing human population requiring food security. Due to its climatic and environmental situation, Africa is also very much affected by infectious agents that are believed to be responsible of up to 25% livestock production losses. Vaccines are one of the best tools to combat pathogenic infectious agents, and more so considering increasing antimicrobial resistance associated with the use of traditional medicines and other drugs. The availability of the appropriate vaccines has however been a challenge on the continent, essentially due to the reducing production capacity of the state-owned vaccine production units, which constitute the main suppliers of livestock vaccine throughout Africa. Besides the decreasing and in some cases, disappearing vaccine production capacity of these institutions, several needs that could have been addressed by improved or new generation vaccines are not being met, while globally the veterinary vaccine segment is one of the fastest growing in the biopharmaceutical industry.

Ways of addressing vaccine adoption in Africa, through increased demand, vaccine availability, access and demand will be discussed.

Biography: Professor Baptiste Dungu is a qualified South African veterinarian, with a PhD in vaccinologist (University of Pretoria). He is one of 6 members of the Scientific Commission of the World Animal Health Organisation (OIE), Paris, France; and a Board member of the IVVN. He has more than 20 years of international experience in vaccine research, development, manufacturing and commercialisation.

Over the past 15 years, he conducted consultancies in more than 20 African countries for different international organizations such as IAEA, FAO, as well as the African Union-IBAR and SADC. Has one patent, more than 30 peer-reviewed articles to my credit, and more than 80 congress contributions and other publications.

Streptococcus suis interactions with B lymphocytes: uncovering the role of IgM

Mariela Segura

Canada Research Chair in Immunoglycobiology of infectious diseases , Director of the Swine and Poultry Infectious Diseases Research Center, Faculty of Veterinary Medicine, University of Montreal, Canada - *mariela.segura@umontreal.ca*

Streptococcus suis causes significant economic losses to the swine industry and raises concerns about animal welfare. This organism is also an emerging zoonotic pathogen and a niche of antimicrobial resistance (AMR) genes of public health concern. In the absence of commercial vaccines, the incidence of disease in pigs is controlled by extensive antimicrobial prophylaxis. From a One Health perspective, vaccines should be a key component of strategies for preventing S. suis disease and AMR threat. S. suis is an encapsulated bacterium, and its thick capsular polysaccharide (CPS) cloaks antigenic proteins on the bacterial surface that would otherwise trigger a protective immune response, thus allowing bacterial evasion of host immunity. Despite the clinical significance of bacterial CPSs, the mechanisms underlying innate and, critically, adaptive immune

responses to encapsulated bacteria have not been fully elucidated. By combining cellular and molecular approaches, our team have showed that S. suis can interfere with optimal antigen-presenting cell (APC) and T cell functions. However, the interactions between S. suis and B cells are largely unknown. Our recent studies indicated that the antibody response plays a large role in immunity against S. suis, with germinal center (GC)-independent but T cell-dependent germline IgM being the major effective antibody specificities. Our results further highlight the importance IgM, and potentially anti-CPS antibodies, in clearing S. suis infections and provide insight for future development of S. suis vaccines.

Biography: Dr. Mariela Segura is full Professor at the Faculty of Veterinary Medicine of the University of Montreal and the Director of the Swine and Poultry Infectious Diseases Research Centre, a strategic research network of Quebec, Canada. Prof. Segura is also the Associate Director of Animal Health & Agriculture of the Canadian Glycomics Network (GlycoNet).

Mariela Segura received her M.Sc. and Ph.D. from the Faculty of Veterinary Medicine of the University of Montreal, where she started her independent research in 2007. She presently holds a Canada Research Chair in Immunoglycobiology of infectious diseases, which are awarded to outstanding researchers acknowledged by their peers as world leaders in their fields. Her laboratory applies multidisciplinary approaches, from biochemistry to cellular and molecular immunology, to dissect the role of bacterial capsular polysaccharides on the immuno-pathogenesis of the disease caused by pathogenic streptococci, such as Streptococcus suis. Dr. Segura is also interested in glycobiology research and carbohydrate-based vaccine development.

Research contributions of Prof. Segura have been recognized by 27 awards and distinctions, a few of which are the UNESCO-L'Oréal Canada for Women in Science Research Excellence Award; the 'Zoetis (Pfizer)' Award of Excellence in Research; the 'Fisher Award' of the Canadian Society of Microbiologists, and the Women of Distinction Award in Science and Technology. She is author or co-author of more than 150 publications, including research articles, review articles and book chapters and over 60 invited lectures worldwide. She has contributed to the training of more than 100 students and research trainees.

Veterinary Immunology: from knowledge to solutions

Samuel Thevasagayam

Bill and Melinda Gates Foundation - Samuel.Thevasagayam@gatesfoundation.org

Biography: Dr. Samuel Thevasagayam leads the program's livestock portfolio, overseeing implementation of the foundation's strategy for animal health, animal production, and animal systems. Before joining the foundation in 2012, Sam spent most of his career within the pharmaceutical industry, working in clinical development, regulatory affairs, business development, and external research alliances.

Sam was responsible for the development and registration of several drugs and vaccines (human and animal) in North American, European, and international markets. Previously, he served as the director of research and development for global alliances for livestock veterinary medicine with GALVmed, a not-for-profit animal health organization. He spent his early career teaching and practicing companion animal internal medicine before spending five years in veterinary virology research at the United Kingdom's Pirbright Institute.

Sam holds a degree in veterinary medicine and surgery from the University of Peradeniya in Sri Lanka, a Ph.D. in veterinary virology for his research on foot-and-mouth disease virus at the Pirbright Institute, and an MBA from the Säid Business School at the University of Oxford. Sam is a fellow of the Royal Society of Biology.

Plenary Presentations

Plenary 1: Local adaptive immunity in pigs – getting the right balance?

Wilhelm Gerner

The Pirbright Institute, Woking, United Kingdom - Wilhelm.Gerner@pirbright.ac.uk

In recent years there was substantial methodological progress to dissect immune responses of livestock species in detail. In this talk I will provide an overview of tools available to dissect T and B cells analyses in pigs. Further I will share findings how a combination of scRNA-seq and multi-dimensional flow cytometry can be used for detailed insights into local effector and regulatory phenotypes of T cells in pigs and their association with B cell and antibody responses in the context of immunisation and infection. This will be placed into a simple inflammation/ immunoregulation model that may help us to identify phenotypic and functional signatures associated with protective immunity.

Biography: Wilhelm Gerner (WG) currently leads the T-cell Biology Group at The Pirbright Institute, UK, and has worked for more than 20 years on the T cell response in pigs. His research contributed to the characterisation of various porcine T cell subsets and their phenotypic and functional changes during differentiation. He has studied T cells in the context of viral (swine influenza A virus, FMDV, PRRSV) and bacterial infections (Actinobacillus pleuropneumoniae, Salmonella Typhimurium). This work also included the analysis of the immune response to vaccination. More recently WG has focused on the phenotypic characterisation of Tfh cells and B cell subsets, including plasma cells, germinal centre B cells and IL-10 producing B cells.

Plenary 2: Immunogenomics and resistance to disease: antiviral immunity in bats

Michelle L. Baker

CSIRO, Health and Biosecurity Business Unit, Australian Centre for Disease Preparedness, Geelong, Vic, 3220 - Michelle.Baker@csiro.au

Bats have been identified as the natural reservoir to an increasing number of emerging and re-emerging zoonotic viruses including the henipaviruses, Nipah and Hendra virus – both members (family Paramyxoviridae). Both Hendra and Nipah are associated with severe neurological and respiratory disease and high mortality rates in humans. However, similar to the response of bats to other viruses, no clinical signs of disease are observed in bats during natural or experimental infections. The long co-evolutionary history of bats with viruses may have shaped the immune system of bats and resulted in unique adaptations for the control of viral replication. To understand how bats coexist with viruses we have developed the Australian black flying fox, the natural reservoir of Hendra virus as a model species for studying virus-host interactions. Analysis of the whole genome of our model bat species has revealed differences in innate immune genes, including

molecules involved in interferon production and signalling and natural killer cells. These molecules provide the first line of defence against infection and shape other arms of the immune response. Functional analysis in vitro and following experimental infection of bats has provided insights into the kinetics of viral infection and host response.

Biography: Dr Michelle Baker is a Principal Research Scientist the Australian Centre for Disease Preparedness. She has a PhD from the University of Queensland and postdoctoral training at the University of New Mexico in the US. Dr Baker's current research is in the area of antiviral immunity, in particular, the innate immune response of reservoir hosts including bats which are hosts to a variety of emerging and re-emerging infectious diseases that affect humans. Her research team has made significant progress in characterizing the immune system of the model bat species, the Australian black flying fox and the responses of bat cells to infection with highly pathogenic viruses including the paramyxovirus, Hendra virus and the filovirus, Ebola virus. More recently her team has developed human 3D cell culture models for studying emerging infectious diseases such as SARS-CoV-2 and testing antivirals.

Oral Presentations

By theme

Theme 1. Tissue Specific Immunity

029 - Characterization of bovine and ovine ileal organoids after polarity exchange

Ambre F Chapuis[1,2], David Smith[1], Dan R.G Price[1], Barbara Shih[2], Marc N Faber[1], Jayne C Hope[2], Jo Moore[1]

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Organoids are three-dimensional (3D) polarized structures composed of different cell types which are capable of self-organization and self-renewal, resembling their organ of origin in architecture and function. They typically self-organize with a basal-out polarity when cultured in a 3D extracellular matrix scaffold and this can present a hurdle for experiments that require access to the apical epithelial surface. Methods to inverse the surface polarity of intestinal organoids have been reported. However, there is little information on how this change of polarity impacts gene expression and cell populations present within the organoids. To address this knowledge gap, we studied apical-out and basal-out intestinal organoids from two different ruminant species. Live imaging and microscopy analysis were carried out to investigate the morphological difference in the organoids after polarity exchange, complemented by staining with specific markers including Ki67 and POU2F3 to explore the cellular composition of the basal-out and apical-out organoids. Overall, apical-out organoids retain a more spherical morphology than their branching basal-out counterpart but no significant change in cell presence was observed. However, for a better understanding of the model viability, bulk RNA sequencing and deep transcriptomic analysis was carried out. We demonstrate that apical-out organoids retain the same gene expression profile as basal-out organoids, including the retention of cell type-specific molecules that evidence the presence of cell growth and mobility. This represents a comprehensive validation of the apical-out organoid model in ruminants and provides further evidence of the usefulness of this model for experiments that require access to the apical surface.

Session 1.1.2 – Saturday 18 November | 10:30

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050 - Induction of antigen-specific secretory IgA in small intestine of newborn piglets after parenteral immunization with retinoic acid-enhanced adjuvant

Gitte Erbs[1], Jeanne Toft Jakobsen[1], Signe Tandrup Schmidt[1], Dennis Christensen[2], Mick Bailey[3], Gregers Jungersen[1]

[1]Statens Serum Institut, Copenhagen, Denmark. [2]Croda Pharma, Frederikssund, Denmark. [3]Bristol Veterinary School, University of Bristol, Bristol, United Kingdom

Mucosal secretory IgA (SIgA) produced by subepithelial plasma cells in the lamina propria is the major antigen-specific defense mechanism against non-invasive mucosal infections. In the small intestine these plasma cells are induced in the Peyer's patches and mesenteric lymph nodes under the influence of retinoic acid (RA), which regulates the expression of specific adhesion molecules that guide the homing of activated lymphocytes to the submucosal effector site via the systemic circulation. Here we immunized neonatal pigs sub-cutaneously with a hybrid protein antigen formulated in RA-containing adjuvant, to hijack the homing of B cells from induction to effector site and induce enhanced intestinal SIgA responses after parenteral vaccination. Using

assays specific for antibody isotypes and secretory component of SIgA, high-dose RA-adjuvant was shown to facilitate a stronger (or faster) IgG and IgA response in serum after primary immunization, and a more than 10-fold significantly increased level of SIgA, but not IgG, in jejunum two weeks after the secondary boost. The correlation between jejunum IgA and jejunum SIgA was high (62%), indicating that IgA measured in jejunum to a large extent reflects locally produced SIgA. Significantly increased IgA or SIgA in bronchoalveolar lavage (BAL) was not observed after RA immunization, which indicates the homing was specific for the intestinal mucosa. We observed, however, a significant correlation between IgA (or SIgA) in jejunum and BAL, indicating a level of commonality in the regulation of antibodies in gut and respiratory system. These results suggest that it may be feasible to induce mucosal immunity by vaccination with adjuvant systems that emulate the RA-driven immunoregulation of GALT inductive sites in draining lymph nodes after parenteral immunization.

Session 1.1.2 – Saturday 18 November | 10:48

088 - Precision-cut lung slices (PCLS) as a platform to study *Mycoplasma hyopneumoniae* interactions with porcine lung tissue

Joanna Myslinska[1], Carin Biel[2], Jon Cuccui[3], Viviane Filor[4], Oliver Gomez-Duran[5], Peter Olinga[2], Rob Noad[1], Andrew Rycroft[1], Dirk Werling[1]

[1]Royal Veterinary College, Pathobiology and Population Sciences, London, United Kingdom. [2]University of Groningen, Groningen Research Institute of Pharmacy (GRIP), Groningen, Netherlands. [3]London School of Hygiene and Tropical Medicine, Glycoengineering and Glycobiology Group, London, United Kingdom. [4]Freie Universität Berlin, Institute of Pharmacology and Toxicology, Berlin, Germany. [5]Boehringer Ingelheim, Bracknell, United Kingdom

Mycoplasmas are prevalent pathogens in pig population, with six species known to colonise swine tissues. *Mycoplasma hyopneumoniae* is the primary pathogen responsible for enzootic pneumonia, a chronic respiratory disease affecting 30-80% of swine and significantly damaging to the pig industry. This highly virulent pathogen is challenging to eradicate due to its ability to evade immune recognition and modulate the immune response, facilitating host invasion. Our study aims to investigate the interaction between *Mycoplasma hyopneumoniae* and cranially-located porcine lung tissue through an in vitro approach. To achieve this, we have successfully adapted the precision cut lung slicing (PCLS) technique. We resuspended *Mycoplasma hyopneumoniae* 232 in Friis medium and incubated it at 37ºC, 5% CO_2 for 48h before infection. Freshly harvested pig lungs were filled with pre-warmed 1.5% agarose while still warm and sliced using the Krumdieck Tissue Slicer. The slices were immediately transferred to pre-warmed tissue culture, incubated overnight, and then transferred to fresh culture. We infected the slices with LPS (1µg/µl) and *Mycoplasma hyopneumoniae* (10^7 CFU/mL), using different medium compositions: serum-free, 2% foetal calf serum (FCS), and 10% FCS. The slices viability was assessed at 0 hours post-infection (hpi), 24hpi, and 48hpi, while IL-1β and TNF-α responses were measured at 24hpi and 48hpi for all conditions. The PCLS model demonstrated optimal viability in serum-free conditions. Additionally, *Mycoplasma hyopneumoniae* exhibited the greatest impact in serum-free composition. The model also revealed a significant IL-1β response induced by Mycoplasma hyopneumoniae but no significant TNF-α response. In summary, our study demonstrates the successful implementation of the PCLS model, which can be employed to investigate *Mycoplasma hyopneumoniae*-induced inflammation in porcine lung. Our findings highlight the importance of serum-free conditions for maintaining slice viability and reveal specific inflammatory response triggered by *Mycoplasma hyopneumoniae*, emphasising the potential of this model for further research in this field.

Session 1.1.2 – Saturday 18 November | 11:06

128 - The role of pathogens and anti-PRRSV immunity in the Porcine Respiratory Disease Complex

Elisa Crisci[1], Andrew R Kick[1,2], Lizzette M Cortes[1], John J Byrne[1], Amanda F Amaral[1], Phillip C Gauger[3], Emily Mahan-Riggs[4], Jeremy Pittman[4], Tobias Käser[1,5]

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The Porcine Respiratory Disease Complex (PRDC) is a multifaceted polymicrobial syndrome that results from a combination of environmental stressors, primary infections (e.g., the immunosuppressive PRRSV) and secondary or opportunistic infectious agents (viruses and bacteria). PRDC causes severe lung pathology leading to reduced performance together with increased mortality rates and production costs in the pig industry worldwide. While previous studies have evaluated the role of PRRSV or selected primary/secondary pathogens, our goal was to perform a comprehensive study correlating both the anti-PRRSV immune response as well as 20 secondary infectious agents with PRDC disease severity. To this end, PRRSV-negative weaners were vaccinated with an MLV and put into a farm with PRDC history. Then, the anti-PRRSV cellular and antibody response was followed pre-vaccination, at 4 weeks post vaccination and during PRDC. In addition, NanoString was used to quantify 20 pathogens within the bronchoalveolar lavage (BAL) at day of necropsy – during PRDC outbreak. Nine pathogens out of 20 were detected in at least one pig and PRRSV was present in 53 out of 55 pigs. During PRDC, the median neutralizing antibody titers against the farm-prevalent PRRSV strain in both BAL and serum ranged from 0-64. The anti-PRRSV interferon-gamma response ranged from absent to very strong (>1,000 spots/million PBMC). Surprisingly, single correlation analyses did not reveal significant correlations between the anti-PRRSV responses or pathogens and PRDC disease severity supporting the multifaceted nature of PRDC. Therefore, future studies will use the collected data to perform more in-depth combinatorial correlation analyses to the role of PRRSV and secondary infectious agents in PRDC.

Session 1.1.2 – Saturday 18 November | 11:24

Theme 2. Immuno-diagnostic

005 - Revealing the sheep immune cell composition using multidimensional flow cytometry

Ezgi Sengun[1], Daan Ophelders[2], Willemijn Hobo[3], Bram van Cranenbroek[1], Marien I. de Jonge[1], Tim Wolfs[2], Renate G. van der Molen[1]

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The sheep model has shown to be important for translational research, studying human disease characteristics and has provided valuable insights in a diverse range of studies on cardiovascular disorders, orthopaedic examination, reproduction and neurodegenerative diseases. Revealing the immune system of sheep holds a

great promise to improve the safety and efficacy of therapeutic agents developed to treat diseases in humans and sheep. However, there is still no detailed assessment of the ovine immune system possible due to the limited availability of commercial antibodies and fluorochrome labels. Therefore, we conjugated unlabeled antibodies in-house to study the sheep immune cell landscape. With our comprehensive multicolor flow cytometry panel, we could identify antigen presenting and lymphocyte populations in adult sheep. The antigen presenting cells (APC) panel consisted of CD11b, CD14, CD16, CD21, CD335, CD206, HLA-DR, CD45, gamma delta(γδ) TCR, live/death markers. The lymphocyte panel contained the following markers: CD45R, CD25, WC1, γδ-TCR, FOXP3, CD5, CD62L, CD4, CD8, CD45 and a live/death marker. After selecting for live CD45+ cells, we can identify B cells (CD21+HLADR+), monocyte (CD11b+CD14+/-CD16+/-), macrophage (CD11b+ CD335-HLADR+CD206+CD16+) and NK cell (CD11b+CD16+CD14-CD335+) populations in the same panel. In addition to live CD45+ cells, we can classify CD5+CD4+ and CD5+CD8+ T cells, regulatory T cells (CD25+FOXP3+), memory T cell subsets (CD45R+/-CD62L+/-) and γδ T cells (γδ TCR+WC1+, +/-FOXP3) in one panel. Presence of multiple lineage markers in these panels can provide more accurate classification of immune cell populations leading to a comprehensive insight into systemic immune responses of sheep. Our approach will also allow to discover novel immune cell subpopulations through bioinformatic tools.

Session 2.2.2 – Sunday 19 November | 10:48

012 - Investigating the biomarker potential and development of lateral flow assays to detect host proteins for improved diagnosis of bovine tuberculosis

Hamza Khalid[1,2,3], Louise Pierneef[3], Anouk van Hooij[3], Zijie Zhou[3], Danielle de Jong[4], Elisa M. Tjon Kon Fat[4], Timothy Connelley[1], Paul L.A.M. Corstjens[4], Annemieke Geluk[3], Jayne Hope[1]

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Bovine tuberculosis (bTB), caused by Mycobacterium bovis, is a globally prevalent infectious disease with significant animal welfare and economic impact. Efficient control measures rely on early diagnosis using tuberculin skin test (TST) and interferon gamma (IFNγ) release assays (IGRAs), followed by culling of positive animals. Difficulties in implementing test-and-slaughter measures in low- and middle-income countries (LMICs) and the underperformance of TST and IGRA in field situations establishes a need to develop improved diagnostics. Here, we aimed to identify biomarker potential of host proteins other than IFNγ; followed by development of up-converting reporter particles (UCP) based lateral flow assays (LFAs). First, sample cohorts of naïve and M. bovis experimentally challenged animals with or without prior BCG vaccination were tested by enzyme linked immunosorbent assay (ELISA). Although serum concentrations were low, the levels of PPDb-specific IL-2, CXCL9, CXCL10 and CCL4, in addition to IFNγ, were significantly higher in M. bovis challenged animals compared to naïve animals. Furthermore, PPDb-specific IL-2, CXCL10 and CCL4 in addition to IFNγ showed DIVA potential, i.e., enabling differentiation of M. bovis infected animals from BCG vaccinated animals. We selected six promising proteins (IFNγ, IL-2, IL-6, CCL4, CXCL9 and CXCL10) and developed UCP-LFAs for their quantitative detection. UCP-LFAs present an accurate but less equipment- and labor-demanding platform than ELISA with proven application for human infectious diseases. In line with the ELISA data, PPDb specific levels of IFNγ, IL-2, IL-6, CCL4 and CXCL9 determined by UCP-LFAs can discriminate M. bovis challenged animals from naïve (AUC range: 0.87-1.00) and BCG vaccinated animals (AUC range 0.97-1.00). We report, for the first time, the potential of UCP technology to detect bovine host proteins. These findings could allow us to develop a robust, user-friendly multi-biomarker test (MBT) for bTB diagnosis.

Session 2.2.2 – Sunday 19 November | 10:30

161 - Identification of novel epitopes capable of differentiating between vaccine and field lumpy skin disease virus

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Lumpy skin disease (LSD) was first identified in South Africa in 1944 and remains endemic. LSD is a notifiable disease by the Department of Agriculture, Land Reform and Rural Development (DALRRD). A live attenuated vaccine (LAV) has been available since the 1960s, and annual vaccination is recommended but it is not a legal requirement. Vaccine coverage is generally low, resulting in annual outbreaks. Virus neutralising antibodies are essential for the immune system of the animal to provide protective immunity, regardless of whether these develop due to vaccination or previous exposure. It is impossible to serologically differentiate an infected from vaccinated animal (DIVA) with current tests. A sero-prevalence study and survey was conducted in Gauteng between 2019 and 2020 with a positivity rate of 39.9 %. The information gathered from farmers did not correlate with the sero-prevalence data, possibly due to natural exposure. A DIVA test will help to manage risks associated with LSD, especially when the vaccination status is uncertain due, e.g., to poor record-keeping. There are 2200 single nucleotide polymorphisms described between the LAV and field viruses. To identify epitopic differences between the LAV and field strains, fragmented-genome phage display technology was used. This gives a global snapshot of the epitopes that induce antibody production by the respective LSDV strains. The OBP vaccine and the Warmbaths field isolate genomes were randomly fragmented by ultrasonication and cloned into a phage display vector, resulting in LSDV peptides displayed on the phage particle as protein IIIV fusions. Sera from OBP vaccinated and Warmbaths infected cattle were used to select peptides from the libraries. After selections, the total output of binding phages were subjected to high throughput sequencing. Differences between peptides selected will hopefully identify unique proteins or peptides that could be used to develop a DIVA assay for sero-surveillance studies.

Session 2.2.2 – Sunday 19 November | 11:06

Theme 3. Innate Immunity

The role of bovine dectin-1 in inducing trained immunity and cross-protection

Dirk Werling, Sam Willcocks, Vivianne Filor, Wolfgang Bäumer, Marie-Christine Bartens, Amanda Gibson

The C-type lectin receptor Dectin-1 was originally described as receptor for β-glucan expressed in myeloid cells, with crucial functions in antifungal responses. However, over time, different ligands both of microbial-derived and endogenous origin have been shown to be recognized by Dectin-1, with the outcomes having profound impact on cytokine production, reactive oxygen species generation and phagocytosis. Nonetheless, tolerant responses have been also attributed to Dectin-1, depending on the specific ligand engaged. Indeed, there is now ample evidence from the human and murine system that Dectin-1 is involved in the development of “trained immunity”, the memory of the innate immune system. Research in the last years has pointed to the broad benefits of trained immunity for host defence and vaccination but has also suggested potentially detrimental outcomes in immune- mediated and chronic inflammatory diseases. However, whereas the induction of adaptive memory is due to the generation of memory lymphocytes, trained immunity is based on changes in metabolic programming.

Here, I will summarise our current understanding of the role of dectin-1 in the bovine system, will show changes in metabolic programming after incubation of bovine macrophages with glucans, and will demonstrate the impact of this re-programming on the tissue level.

Session 1.1.1 – Saturday 18 November | 08:30

068 - Contributions of porcine gamma-delta T cells to antiviral immunity: An investigation into Toll-like receptor expression and potential cytotoxic activity

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Gamma-delta (γδ) T cells represent a prominent lymphocyte subset in pigs. However, developmental changes, antigen recognition, cell migration, and their contributions to pathogen clearance remain largely unknown. Thus, we aim to characterize porcine γδ T cells and their antiviral mechanisms. Toll-like receptors (TLR) are key receptors for recognizing pathogens by innate immune cells, and our experiments revealed TLR transcript in γδ T cells, with the highest expression observed for TLR7 and 8. Using flow cytometry, kinome analysis and inhibitory experiments, we confirmed that the TLR7/8 expression by γδ T cells is indeed functional and serves as a co-stimulatory signal, likely signaling through the MyD88-dependent pathway. Moreover, γδ T cells showed both conserved and age-specific responses highlighting the need for comparative studies. Based on this data, porcine γδ T cells could be involved in an antiviral response by recognizing viral RNA through TLR7/8 and subsequently enhancing the adaptive immune response by producing IFNγ at the site of infection. Besides cytokine production and recognition of PAMPs, human γδ T cells are known to have potent cytotoxic activities against infected or cancerous cells, but no data are available concerning porcine γδ T cells. Therefore, we further investigated γδ T cells' direct interactions with porcine alveolar macrophages (PAMs), which were either mock-infected or infected with swine Influenza A virus (swIAV). Unstimulated or IL-2/IL-15 stimulated γδ T cells were cocultured with PAMs at different effector: target ratios, and cytotoxicity was measured using a flow-cytometry based assay. Preliminary data suggest that γδ T cells mediated cytotoxicity exist against uninfected PAMs (up to 40%) but that γδ T cells exhibit poor cytotoxic responses against swIAV-infected targets. This might indicate a swIAV-mediated modulation of γδ T cells recognition and lysis of infected cells. However, these preliminary observations require further confirmation, and mechanistic studies are ongoing.

Session 1.1.1 – Saturday 18 November | 09:06

116 - Virulent African horse sickness virus serotype 4 interferes with the innate immune response in horse peripheral blood mononuclear cells ***in vitro***

Erika Faber[1], Selaelo Ivy Tshilwane[2], Mirinda Van Kleef[1], Alri Pretorius[1]

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African horse sickness (AHS) is an insect-transmitted, infectious but noncontagious disease of equids that can cause more than 90% mortality in susceptible horses. The disease is caused by African horse sickness virus (AHSV), a member of the family *Reoviridae*. There are no specific antiviral treatments for horses suffering from AHS. The disease is controlled by preventative vaccination of susceptible horses in endemic areas with the polyvalent AHSV live attenuated vaccine. Since attenuated vaccines are not compatible with DIVA and due to various bio-safety concerns, many studies are trying to develop safer new generation AHS vaccines; but none has been commercialized to date. To gain knowledge about the host immune response and the immune

evasion strategies the virus employs, our team used transcriptome analysis of RNA sequences to characterize the global immune responses induced in horse PBMC after 24 h by the attenuated AHSV serotype 4 (attAHSV4) vaccine in vivo and a virulent AHSV4 (virAHSV4) field isolate *in vitro*. The lack of immune evasion during the attAHSV4 immune responses resulted in well-developed and regulated innate and adaptive immune responses. In contrast, after translation, proteins of virAHSV4 interfere with the innate immune response. Viral interference with type I and type III IFN responses resulted in an impaired innate immune response that was not able to eliminate virAHSV4-infected PBMC and gave rise to prolonged expression of pro-inflammatory cytokines and chemokines during the virAHSV4 primary immune response. This might give rise to an excessive inflammatory response that causes immunopathology, which could be a contributing factor to the pathogenesis of AHS in a naïve horse. Viral interference was overcome by the fast kinetics and increased effector responses of innate immune cells due to trained innate immunity and memory T cells and B cells during the virAHSV4 secondary immune response.

Session 1.1.1 – Saturday 18 November | 09:24

Theme 4. Adaptive Immunity

019 - PD-L1 blockade enhances T-cell response to vaccination in calves

Tomohiro Okagawa[1], Satoru Konnai[1,2,3], Hayato Nakamura[2], Otgontuya Ganbaatar[2], Yamato Sajiki[2], Kei Watari[2], Haruka Noda[4], Mitsuru Honma[4], Yukinari Kato[5,6], Yasuhiko Suzuki[1,3,7,8], Naoya Maekawa[1], Shiro Murata[1,2], Kazuhiko Ohashi[1,2,9]

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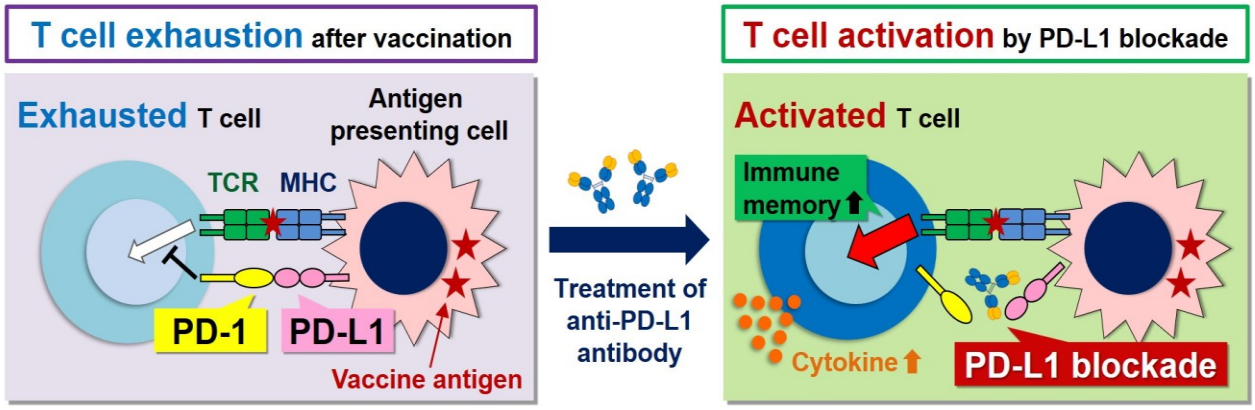
Background: Vaccination is essential to protect against various infectious diseases in calves. T cells is responsible for cellular immunity to vaccination with attenuated live viruses. However, interactions between programmed death-1 (PD-1) and PD-ligand 1 (PD-L1) cause functional exhaustion of T cells, thereby attenuating the induction of effector and memory T cells by vaccination. Previously, we have developed a rat-bovine chimeric anti-bovine PD-L1 blocking antibody (Ab) and have demonstrated that blockade of the PD-1/PD-L1 interaction reactivates T-cell responses in cattle.

Objective: The present study aimed to examine the potential utility of PD-1/PD-L1-targeted immunotherapy in enhancing T-cell responses to vaccination in calves.

Methods: Calves (*n*=6 in each group) were inoculated with two doses of a hexavalent live-attenuated viral vaccine against bovine respiratory diseases (Calfwin-6) in combination with or without anti-PD-L1 Ab (Boch4G12). PD-1 expression on T cells in peripheral blood mononuclear cells (PBMCs) were measured by flow cytometry before and after the prime and booster vaccinations. PBMCs were cultivated with UV-inactivated whole viral antigens (BRSV or BVDV-1), and numbers of antigen-responding T cells and cytokine response were analyzed by flow cytometry and ELISA before and after the vaccinations.

Results: PD-1 expression was upregulated in $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ T cells in calves after a booster vaccination. The expression analysis of CD25 and CD69, markers of lymphocyte activation, in the stimulated PBMCs enabled tracking of T-cell responses to vaccinations. The frequency of $CD25^+CD69^+$ cells was increased in $CD4^+$, $CD8^+$, and $\gamma\delta TCR^+$ T cells stimulated with the viral antigens after a booster vaccination with PD-L1 blockade. In addition, IFN-γ responses to the viral antigens were increased following the booster combinatorial vaccination.

Conclusion: The blockade of the PD-1/PD-L1 interaction enhances T-cell responses induced by vaccination in calves. This finding indicates the potential utility of anti-PD-L1 Ab in improving the efficacy of current vaccination programs.



Session 1.1.1 – Saturday 18 November | 15:25

023 - Diet and defences: low affinity antibodies in bats are affected by food quality

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Bats are reservoirs for zoonotic viruses, but we do not understand how bats develop their antibody repertoire. Experimental infections suggest bats generate low neutralizing titers, but field studies have found bats with high serum antibody titers, often during times of food stress events. Using Jamaican fruit bats (JFBs) and BALB/c mice, we compared titers and B cell receptor (BCR) diversity of JFB and mice in response to T-dependent and T-independent antigens. We then manipulated the diet of JFB bats and infected them with H18N11 influenza A virus. Bats generated a less robust antibody response and possessed more BCR mRNA diversity compared to mice. However, withholding protein from JFBs improved serum titers to H18N11 HA and reduced BCR mRNA diversity. Our results suggest T-cell help to B cells is dampened in bats resulting in low affinity antibodies, but this phenotype can be manipulated with dietary changes.

Session 1.1.1 – Saturday 18 November | 15:43

063 - Exploring the therapeutic potential of canine TGF-β decoy receptor for melanoma that reverses TGF-β1–mediated immunosuppression

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Cancer cells use various mechanisms to evade the host immune system. Transforming growth factor (TGF)-β1 is an immunosuppressive cytokine that induces the differentiation of regulatory T (Treg) cells. Since TGF-β1 is involved in immune evasion mechanism in cancer, the inhibition of the TGF-β1 signaling pathway leads to enhanced antitumor responses and can suppress cancer progression and metastasis. However, there is no report on the TGF-β1 blockade therapy in canine cancers. We first examined the TGF-β1 expression in canine cancers and its effects on the suppression of canine immune cells. TGF-β1 production was observed in the culture supernatant of several canine melanoma cell lines, and serum TGF-β1 levels were elevated in dogs with metastatic oral malignant melanoma. Interestingly, the addition of recombinant TGF-β1 to canine peripheral blood mononuclear cell (PBMC) culture decreased the production of Th1 cytokines and promoted $CD4^+CD25^+Foxp3^+$ lymphocyte (Treg cell) differentiation. Thus, reversal of the immunosuppression by the TGF-β1 blockade may become a novel therapeutic strategy for canine cancers.
Secondly, as a potential TGF-β1 blocker for canine cancer treatment, a decoy receptor for TGF-β, TGF-βRII-Ig, was prepared as a fusion protein of the extracellular region of canine TGF-β receptor II (TGF-βRII) and the Fc region of canine IgG-B. The binding of TGF-βRII-Ig to TGF-β1 was confirmed by ELISA. The addition of TGF-βRII-Ig to the TGF-β1-treated PBMC culture increased the Th1 cytokines production and reduced the differentiation of Treg cells, suggesting that TGF-βRII-Ig competitively inhibits the immunosuppressive effects of TGF-β1 and thereby enhances T-cell mediated immune responses. In conclusion, TGF-βRII-Ig is a novel candidate biologic for the treatment of canine cancers that produce TGF-β1 in the tumor microenvironment. The antitumor efficacy of the TGF-β1 blockade therapy should be further investigated in clinical studies involving dogs with cancers.

Session 1.1.1 – Saturday 18 November | 16:15

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087 - Transwell cultures of lymph node derived cells for the *in vitro* monitoring of adaptive immune responses in pigs

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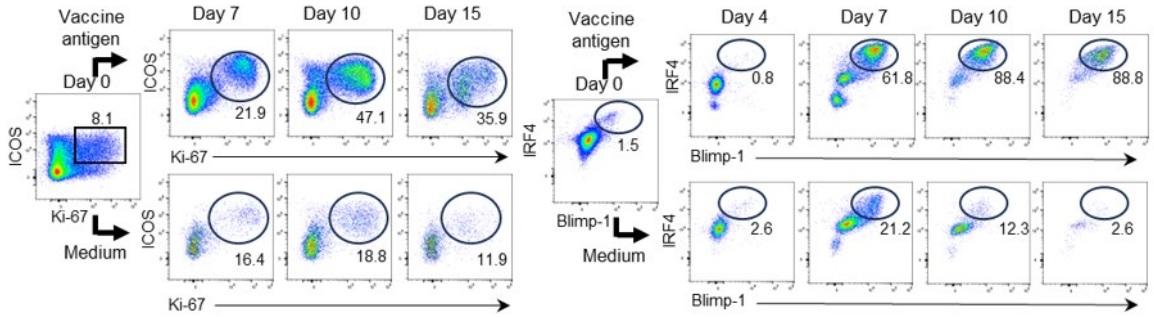
In recent years, there have been growing efforts to develop in vitro models for components of the immune system. In 2020, one report showed that transwell cultures allow the development of human tonsil organoids which show prolonged recall responses of T and B cells and the formation of structures that resemble germinal centres (https://doi.org/10.1038/s41591-020-01145-0). We adapted this system using cell preparations from porcine lymph nodes. Pigs were immunised twice with an inactivated swine influenza A virus vaccine (Ceva RESPIPORC FLUpan H1N1) and cells isolated from lymph nodes draining the trapezius muscle to which the vaccine had been applied. Transwell cultures from cells isolated 21 days post-secondary vaccination were established and restimulated with vaccine antigen. T and B cell activation was investigated by flow cytometry and production of H1N1-specific antibodies by ELISA. Cells were harvested 4, 7, 10, 15 and 21 days after the beginning of the cultivation period. For T cells, also IFN-γ ELISpot analyses were performed from isolated cells at day 0, which were stimulated overnight with vaccine antigen. Although IFN-γ ELISpots are considered as a sensitive tool for studying T cell responses to vaccines, we observed only marginal responses above the medium control. In contrast, a substantial expansion of CD4 T cells with an activated phenotype (CD3+CD4+ICOS+Ki-67+, Figure, left panel, pre-gated on CD3+CD4+) and plasma cells (CD79α/Pax5+/-IRF4highBimp-1+, Figure, right panel, pre-gated for CD79α+ and/ or Pax5+) was observed in transwell cultures

following vaccine antigen restimulation, compared to medium controls. Responses peaked at day 10 of in vitro cultivation, after which cell numbers started to decline. ELISA analyses are currently ongoing. These findings suggest that lymph node transwell cultures are a powerful tool to investigate recall responses to vaccine antigens, which otherwise would be considered as weak or absent.



Session 1.1.1 – Saturday 18 November | 16:33

Theme 5. Immunology of viral diseases

225 - Bovine Viral Diarrhoea Virus (BVDV) preferentially infects arginase-producing bovine monocyte-derived macrophages

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Infection of immune cells by the Bovine Viral Diarrhea Virus (BVDV) has a profound effect on innate immunity, however, the susceptibility of the different phenotypes of macrophages in BVDV infection has not been explored. In this study, we evaluated the interaction between different BVDV strains and monocyte-derived macrophages. Monocyte-derived macrophages (Mo-Mϕ) were collected from healthy cattle and polarized to an MI or M2 state by using LPS, INF-g, IL4 or azithromycin. Arginase activity quantitation was used as a marker of the M2 Mo-Mϕ spectrum. We found a significant association between arginase activity and the replication rate of BVDV strains of different genotypes and biotypes in vitro. Macrophages were then polarized in vivo, by treating calves with azithromycin. This treatment induced Mo-Mϕ of the M2 state that produced high levels of arginase. Mo-Mϕ from azithromycin-treated animals supported higher replication of BVDV associated with an increased arginase activity on day seven post- treatment compared to non-treated animals, constituting a promising model for the study of the Mϕ phenotypes. Mo-Mϕ from pregnant dams and calves produced higher arginase levels than those measured in non-pregnant adult animals, showing that the animal's physiological state can impact the macrophage's phenotype which can, in turn, affect the BVDV infection rate. Altogether, our results verified for the first time that arginase-producing, alternatively activated bovine macrophages support enhanced BVDV replication in vitro and ex vivo and that azithromycin applied to cattle can polarize Mϕ to an M2 state. Understanding the impact of the bovine macrophage's phenotype in BVDV infection may unveil a possible leading role of these cells in establishing an immune-suppressive state and promote further studies to dissect the role of macrophages phenotype, consequential from the animal's physiological state, in the outcome of bovine viral diseases.

Session 1.3.1 – Saturday 18 November | 08:30

057 - Cyclooxygenase (COX)-2 inhibitor inhibited porcine reproductive and respiratory syndrome virus (PRRSV) type 2-induced M2 macrophage polarization

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Alteration of macrophage polarization by viruses has been linked to enhanced viral infectivity and viral pathogenesis. We previously observed that PRRSV-2 triggered M2 macrophage polarization, resulting in suppression of macrophage activities and increased PRRSV replication in the culture. It was hypothesized that inhibiting PRRSV-2-induced M2 macrophage polarization might restore macrophage immunological competences and reduce virus production. Previous report suggested that a cyclooxygenase (COX)-2 inhibitor could switch macrophage polarization from M2 to M1. To explore the potential immunomodulatory effect of COX-2 inhibitor on PRRSV-2-induced M2 macrophage polarization, porcine monocyte-derived macrophages (MDM) were *in vitro* inoculated with PRRSV-2 (strain 01NP1, Thai isolate) or mock-infected MARC-145 cell lysate (mock). The MDM were treated with 50 ng/ml of COX-2 inhibitor at different time points, including 12 hours prior to, simultaneous to, or 12 hours following virus inoculation. At 48 hours following virus inoculation, the cultured MDM and cell supernatants were collected and subjected to immunological and virological analyses, respectively. The results indicated that treatment with COX-2 inhibitor inhibited PRRSV-2-induced M2 polarization through inhibition of M2 signaling proteins (pSTAT6 and pIRF4) and arginase (ARG) expressions. Moreover, treatment with cyclooxygenase-2 inhibitor decreased productions of the immunosuppressive cytokines, including IL-1Ra and IL-10, by the PRRSV-infected MDM. Interestingly, addition of COX-2 inhibitor also reduced viral loads in the culture supernatants. The inhibitory effects of COX-2 inhibitor were prominently observed when added before, or at the time of PRRSV inoculation. The results from this study demonstrated potent inhibitory effects of COX-2 inhibitor on PRRSV-2-induced M2 macrophage polarization and viral replication, which may lead to development of effective disease intervention in the future.

Session 1.3.1 – Saturday 18 November | 08:48

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072 - A systems immunology approach to identify protective and detrimental innate and adaptive immune responses following ASFV infection of pigs

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African swine fever is a deadly disease of wild boars and domestic pigs with a massive negative economic impact in affected areas. The development of an effective and safe vaccine is hindered by a lack of understanding of immune mechanisms underlying protection in susceptible species. The virus targets macrophages and can lead to a catastrophically dysregulated innate immune stimulation. In the present study we have employed a systems immunology-based analysis of pigs infected with the moderately virulent "Estonia 2014" ASFV strain. Recorded parameters included, clinical scores, viremia and viral loads, systemic cytokines, blood transcriptional module analyses, antibody levels, ASFV-specific CD4 and CD8 T-cell activation in terms of TNF-α and IFN-γ production. Perturbation and correlation analyses permitted the dissection of the time-dependent activation processes correlating with protection. Our findings indicate that a shift of the innate response from an unregulated inflammation to an early but controlled interferon type I response was associated with dendritic cell activation, a strong activation of CD4 T helper cell response and protective

immunity. If such responses were weak, aberrant, and persistent sepsis-like inflammation dominated and this was associated with decompensation and severe life-threatening disease. The identified correlates of protection will be helpful in evaluation of vaccine safety and efficacy, and in the identification of viral, host and environmental factors contributing to ASFV virulence.

Session 1.3.1 – Saturday 18 November | 09:06

107 - Modified-live virus vaccination induces heterologous immunity against different type-2 PRRSV strains

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Background: Cross-protection against heterologous strains is a major hurdle of vaccines against PRRSV – the porcine reproductive and respiratory syndrome virus. Heterologous vaccine efficacy relies on the induction of both humoral and cellular immunity that reacts against various PRRSV strains.

Objective: Thus, this study investigated vaccine efficacy and immunogenicity of the Prevacent modified live virus (MLV) vaccine against four type-2 PRRSV strains.

Methods: Sixty weaners were divided into five MOCK- and five MLV-vaccinated groups. After four weeks, each of these groups were challenged for two weeks with MOCK or one of four PRRSV-2 strains – NC174, NADC20, NADC30, or VR2332. Heterologous vaccine efficacy was assessed by lung pathology and viremia. Heterologous vaccine immunogenicity was determined via nasal swab IgA, serum IgG and neutralizing antibody levels, and a detailed T-cell response analysis – proliferation, IFN-γ production, and differentiation of CD4, CD8, and TCR-γδ T cells.

Results: Vaccination showed heterologous efficacy against VR2332 (reduced viremia), and NADC20 and NADC30 (reduced lung pathology and viremia). Vaccination also induced a strong systemic IgG response and increased the number of animals with neutralizing antibody titers against VR2332, NADC20, and NADC30. Vaccination also improved the heterologous T-cell response: Vaccinated animals not only had a higher frequency of memory/effector CD4 T cells but also an improved heterologous CD4 and CD8 IFN-γ response against NC174, NADC20, and NADC30. Correlation analyses showed that the systemic IgG levels and the CD4 T-cell response were the best immune correlates of protection.

Conclusion: Overall, the MLV vaccine Prevacent elicited various degrees of both vaccine efficacy and immunogenicity against different heterologous PRRSV-2 strains. The determination of immune correlates of protection for PRRSV can on the one side strongly improve evaluation of vaccines against emerging strains and on the other side facilitate vaccine development.

Session 1.3.1 – Saturday 18 November | 09:24

146 - Characterization of foot-and-mouth disease virus cross-serotypes reactive and neutralizing antibodies

Angela W Steyn[1], Helen Duyvesteyn[2], Kristel Ramirez Valdez[1], Elena Lokhman[1], Sylvia Crossley[1], Kevan Hanson[1], William Mwangi[1], Eva Perez Martin[1], Claudine Porta[2], Liz Fry[2], Nick Knowles[1], Cristina Faralla[3], Katherine J Lam[3],

Roisin McComb[3], Sherie K Duncan[3], Bryan Charleston[1], Ray Owens[4], David Stuart[2], Marie Bonnet-Di Placido[1], John A Hammond[1]

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Cattle are susceptible to foot-and-mouth disease virus (FMDV), which causes a highly contagious and devastating disease in livestock and poses a substantial threat to food security in Asia, Africa, and the Middle East. Neutralizing antibodies primarily mediate protection against the disease, and vaccine effectiveness is hindered by FMDV cross-serotype antigenic diversity. To identify cross-reactive neutralizing epitopes, we sequentially vaccinated cattle with FMDV antigens from four serotypes and characterized vaccine-mediated B cell and antibody responses. Single FMDV-specific IgG-secreting B cells were isolated and 143 antibody heavy and light chain pairs were sequenced, 50 of which were selected and expressed as recombinant cattle IgG1. Following assessment of avidity by bio-layer interferometry (BLI) and neutralization by virus neutralization test (VNT), we identified 13 single-serotype specific, eight bi-serotype specific, and three broadly neutralizing tri-serotype specific antibodies for O, A, and Asia serotypes. Cryo-electron microscopy revealed that the three tri-serotype specific antibodies bind to conserved and overlapping epitopes of O, A, and Asia serotypes. Our study provides insight into the antibody response to FMDV vaccination and identifies cross-serotype neutralizing epitopes. These findings may inform the development of improved vaccines that elicit broad-spectrum protection against multiple FMDV serotypes.

Session 1.3.1 – Saturday 18 November | 09:42

010 - Avidity ELISA as an alternative to the Virus Neutralization Test to assess Foot-And-Mouth Disease vaccine-induced antibody responses in buffaloes (Bubalus Bubalis)

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Buffaloes are considered a reservoir of foot and mouth disease virus (FMDV) in the carrier state with the potential to spread the virus in the field. They are compulsorily vaccinated in many countries as part of official control programs. Vaccine-induced antibodies in buffaloes are currently evaluated using either the virus neutralization test (VNT) or by liquid-phase blocking ELISA (LPBE). Both assays present flaws. The VNT gives information on the biological activity of antibodies but is cumbersome and has low throughput. On the other hand, the LPBE measures total FDMV-specific antibodies and have not been validated for buffaloes´ samples. Previous data from our laboratory demonstrated that the vaccine- induced antibodies assessed by the LPBE yielded low specificity with buffaloes' samples. In contrast, a single-dilution avidity ELISA (AE) aimed to detect high-avidity antibodies against exposed epitopes, combined with an indirect ELISA (IE) to assess IgG levels, produced more reliable results.

Here we analysed for the first time the kinetics of the antibodies induced by vaccination in two different buffalo herds (n=91) over 120 days using AE, IE, LPBE, and the VNT. Kinetics were similar in the different assays, with an increase of antibodies between 0- and 14-days post-vaccination (dpv) which were maintained thereafter. VNT and AE results were concordant (Kappa value: 0.76), and both assays revealed

a decay in the antibody response in calves with maternal antibodies at 90 and 120 dpv, which was not evidenced by the LPBE. These results show that kinetics of antibody responses to FMD vaccination are similar in buffalo and cattle, and support the use of indirect ELISA assays, in particular Avidity ELISA, as alternatives to the VNT for vaccine-immunity monitoring irrespectively of the animal´s passive or active immune status.

Session 3.2.2 – Monday 20 November | 10:30

190 - African Swine Fever: Cellular and Humoral Immune Response of pigs vaccinated with Live Attenuated Strain

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Background: African swine fever virus (ASFV) is a large and structurally complex virus with a large dsDNA genome encoding for more than 150 genes. Live attenuated virus strains can induce protection in domestic swine against disease produced by homologous virulent parental viruses. The roles of the different immune mechanisms induced by the attenuated strains in protection still needs to be understood. The technical difficult to access the cellular and humoral immune response in addition to infrequent recombinant live attenuated strains availability make the immune studies challenged. Here we present methods to access results from cellular and humoral immune response. In addition, we presented a novel methodology to detect virus neutralizing antibodies based on the reduction of virus infectivity of a Vero cell adapted ASFV strain.

Methods: A total of 84 sera from pigs that were inoculated with two different ASFV attenuated mutants: ASFVG-DI177L and ASFVG-D9GL/ DUK. Indication of cellular immune response was performed by flow cytometry. Detection of ASFV specific antibody was performed with In-house ELISA. Virus neutralization was accessed in a Vero-adapted ASFV growth.

Results: Antibodies against ASF antigens is detected as early as 11 days post inoculation with attenuated strains. Recall response of IFN-gamma to ASF antigens is required to survival at 28 days post vaccinated. The humoral immune response aspects demonstrated a high association between the presence of virus neutralizing antibodies and protection in eighty-four animals immunized with the recombinant vaccine candidates ASFV-G-D9GL/DUK or ASFV-G-DI177L.

Conclusion: This is the first time which is demonstrated an association between virus neutralizing antibodies and protection against virulent challenge, in such a large number of experimental individuals. Therefore, both, cellular and humoral response are required to survival of pigs challenged with wild-type Georgia strain.

Session 3.2.2 – Monday 20 November | 10:48

208 - Peste des petits ruminants virus activates goat dendritic cells but impairs antigen presentation

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Peste des petits ruminants (PPR) is a WOAH notifiable disease affecting sheep and goats caused by Peste des petits ruminants virus (PPRV), a morbillivirus of the Paramyxoviridae family. PPRV infection induces

immunosuppression, which can cause animal death due to opportunistic infections. In general, the disease is more severe in goats than in sheep, but the mechanisms that explain this phenomenon are still unknown. Dendritic cells (DC), which are central to mounting adequate immune responses to pathogens, can be targeted by PPRV in sheep. In the present work, we assessed PPRV effects on goat DC. Monocytes were obtained from caprine peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation and antibody magnetic sorting. Monocytes were differentiated into immature monocyte-derived DC (iMoDC) with growth factors for 72h and matured with the TLR7/8 agonist R848 for 24h. To study the effect of the virus, iMoDCs were infected after 48h differentiation and surface marker expression, phagocytosis and antigen presentation assessed. Goat MoDC expressing the classical MoDC markers (MHCI, MHCII, CD1, CD1w2, CD80, CD86, CD40, CD209, CD11b, CD11c and CD172a) and typical morphological characteristics were obtained using our culture method. PPRV infected caprine MoDC and this led to the upregulation of maturation markers CD40, CD80 and CD86. Moreover, the phagocytic capacity of infected MoDC was decreased when compared to mock counterparts, which further indicated that the infection produced MoDC maturation. Mixed lymphocyte reaction assays however showed that PPRV infection reduced caprine MoDC capacity to stimulate CD4+ and CD8+ T cell proliferation. PPRV can therefore target goat DC to disrupt their ability to present antigen. In this study, we established a protocol to obtain functional goat MoDC to study viral infections. We found that PPRV infection affected the characteristics of these caprine DCs, producing a partial maturation status that is inefficient at antigen presentation.

Session 3.2.2 – Monday 20 November | 11:06

209 - Comprehensive immune profiling reveals that arbovirus infection activates immune checkpoints during acute t cell immunosuppression

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Bluetongue virus (BTV) is an arbovirus transmitted by the bite of infected Culicoides midges that affects domestic and wild ruminants producing great economic losses. The infection induces an IFN response, followed by an adaptive immune response that is essential in disease clearance. BTV can nonetheless impair IFN and humoral responses. The main goal of this study was to gain a more detailed understanding of BTV pathogenesis and its effects on immune cell populations. To this end, we combined flow cytometry and transcriptomic analyses of several immune cells at different times post-infection (pi). Four sheep were infected with BTV serotype 8 and blood samples collected at days 0, 3, 7 and 15pi to perform transcriptomic analysis of B-cell marker+, CD4+, CD8+, and CD14+ sorted cells. The maximum number of differentially expressed genes occurred at day 7pi, which coincided with the peak of infection. KEGG pathway enrichment analysis indicated that genes belonging to virus sensing and immune response initiation pathways were enriched at day 3 and 7 pi in all 4 cell population analyzed. Transcriptomic analysis also showed that at day 7pi T cell exhaustion pathway was enriched in CD4+ cells, while CD8+ cells downregulated immune response initiation pathways. T cell functional studies demonstrated that BTV produced an acute inhibition of CD4+ and CD8+ T cell activation at the peak of replication. This coincided with PD-L1 upregulation on the surface of CD4+ and CD8+ T cells as well as monocytes. Taken together, these data indicate that BTV exploits the PD1/PD-L1 immune checkpoint to impair T cell responses. These findings identify several mechanisms in the interaction between host and BTV, which could help develop better tools to combat the disease.

Session 3.2.2 – Monday 20 November | 11:24

222 - Bluetongue virus disrupts the type-I interferon response by interfering with the cGAS pathway

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Background and Objectives: Bluetongue virus (BTV) is a double-strand RNA virus of the *Orbivirus* genus and the *Reoviridae* family. Although BTV has been described to antagonize IFN signalling, the mechanism by which the virus interferes with IFN-I induction has not been well described. cGAS is a DNA sensing cellular receptor that was understood to respond to pathogen and host derived DNA. However, recent studies have shown that some RNA viruses are capable of activating this pathway, inducing IFN-I production. The aim of this research is to study potential interactions between BTV and cGAS pathway as a possible mechanism of inhibition of IFN-I induction.

Material and Methods: Interferon reporter assays were carried out using HEK-293T cells expressing firefly luciferase under the control of an IFN-ß promotor (293T-IFNß). IFN-ß promotor induction of cells transfected with a plasmid containing the sequence of BTV-NS3 protein was measured using the neolite luminescence reporter gene system. To study potential cGAS or STING degradation, 293T cells were transfected with pCMV-Lenti-cGAS-HA, pcDNA3.1-STING and pIRES-BTV-NS3 or pIRES-BTV-NS4 or infected with BTV-8. Cell lysates were analyzed via SDS-Page and immunoblotting. To evaluate interferon production, immortalized sheep thymus cells were infected with BTV and stimulated with E.coli DNA or MVA. 16 hours post-stimulation, cell lysates were collected for nucleic acid extraction using RNeasy Micro Kit, which were then analyzed by qPCR using specific primers for sheep IFNa and ISG15 genes.

Results: BTV infection inhibits DNA-induced type-I IFN transcription in sheep cells. This inhibition correlates with cGAS degradation during infection. BTV-NS3 protein has been identified as the protein responsible for cGAS degradation, via an autophagy-dependent mechanism in which ubiquitinated NS3 binds to cGAS.

Conclusion: We describe a mechanism by which bluetongue antagonizes the interferon response. During infection, IFN-I induction is hindered due to cGAS degradation mediated by NS3 protein through an autophagy-dependent mechanism.

Session 3.2.2 – Monday 20 November | 11:42

Theme 6. Immunology of bacterial diseases

030 - Chitosan hydrogel induces immune cell recruitment in the bovine mammary gland and increases cure rates of intramammary infections caused by ***Staphylococcus*** spp.

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Bovine mastitis is the most common and costly disease affecting dairy cattle. *Staphylococcus* spp. are the most frequent bacterial isolates of difficult-to-treat intramammary infections. Although antibiotics are the mainstay of treatment, their indiscriminate use has given rise to multiresistant bacteria. Previously, we reported that chitosan (Ch) had important antimicrobial and antibiofilm activity, either alone or combined with antibiotics. Moreover, we found that Ch presented *in vitro* immunostimulatory activity on infected macrophages, improving microbicidal activity with a significant increase in nitric oxide and reactive oxygen species production. This study aimed to evaluate *in vivo* its intramammarily immunostimulant activity, and its efficacy as a drycow therapy alone or combined with cloxacillin. Ch hydrogel (5mg/mL) or Control formulation were applied intramammarily in healthy udders (n=16 per group), and milk was assessed every 24 h in terms of somatic cell count (SCC) and leukocyte subpopulation (flow cytometry). We found that Ch induced leukocyte migration (more than 15-fold), with strong neutrophil and macrophage recruitment in the first hours, while high T-lymphocyte recruitment was observed after 72 h, compared to the control ($p<0.001$). We then evaluated formulations of Ch alone (F4), Ch with 600 mg of cloxacillin (F1), Ch with 120 mg of cloxacillin (F2), and a commercial dry-off therapy formulation as the control (n=40 per group). SCC and a microbiological analysis were performed in each quarter before drying and for 4 weeks after calving. The bacteriological cure rates for F1, F2, and F4 were respectively 68%, 62%, and 55%, while that of the control was 36%. The cure rate against *Staphylococcus* spp. was 100% for the Ch-based formulations and 33% for the control. These data suggest that Ch may be a promising strategy for the treatment of intramammary infections by allowing stimulation of mammary tissue, which could reduce the use of antibiotics in food-producing animals.

Session 1.3.2 – Saturday 18 November | 10:30

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156 - The impact of age on vitamin D receptor expression, vitamin D metabolism and associated cytokines in ex vivo *Rhodococcus equi* infection of equine alveolar macrophages

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Rhodococcus equi (*R. equi*) is an intracellular bacterium that causes morbidity and mortality in young foals. Survival and replication within alveolar macrophages (AMϕ) are the hallmarks of *R. equi*'s pathogenicity. The vitamin D receptor (VDR) and its ligand, the active vitamin D metabolite $1,25(OH)_2D$, are important in the immune response to intracellular bacteria, regulating cytokine production in infected human AMϕ. The immunomodulatory role of the vitamin D/VDR pathway in equine leukocytes is unknown. Our objective was to determine if age would impact synthesis of $1,25(OH)_2D$, VDR expression, and regulation of vitamin D-associated cytokines in an *ex vivo* model of *R. equi* infection in equine AMϕ. AMϕ were collected from ten healthy foals at 2-, 4- and 8-weeks old and from nine healthy adult horses once via bronchoalveolar lavage. AMϕ were mock infected (CONTROL) or infected with a virulent laboratory strain of *R. equi* for 7 days (INFECTED). VDR expression was determined via RT-qPCR in cell lysates. $1,25(OH)_2D$ and cytokines were measured in cell supernatant by immunoassays. VDR expression was impacted by age ($P=0.001$) with the highest expression in expression in cells from 8-week-old foals. Relative VDR expression was lower in INFECTED cells from adults compared to CONTROL ($P=0.002$). There was no effect of age or infection for $1,25(OH)_2D$ ($P>0.37$). Vitamin D-associated cytokine production was impacted by both age and

infection for TNFα and IFNγ (P<0.027). The proportion of samples producing detectable IL-1β, IL-10 and IL-17 was higher in INFECTED compared to CONTROL samples (P=0.031). These data demonstrate that there are age-associated changes in VDR expression and related cytokine production in equine AMϕ in response to *R. equi* infection. Further research is needed to elucidate the immunomodulatory role of the vitamin D pathway in the horse.

Session 1.3.2 – Saturday 18 November | 10:48

046 - Modelling immune responses of cattle to mycobacteria using magnetic bioprinted granulomas

Gesa Krueger[1], Brahmaiah Meesaragandla[1], Shah Faisal[2], Ulrike Zedler[2], Gabriele Stooß[2], Stefan HE Kaufmann[3,4,5], Björn Corleis[2], Mihaela Delcea[1], Anca Dorhoi[2,1]
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Tuberculosis (TB) remains a threat for human and livestock health. Mycobacteria causing TB are host-adapted pathogens which occasionally spillover to other species. Mycobacterium bovis causes bovine TB, a well-known zoonosis. Mycobacterium tuberculosis (Mtb) is adapted to humans, may trigger symptomatic infection in cattle yet these show resistance to Mtb experimental challenge. A hallmark of TB in all hosts are multicellular tissue lesions termed granulomas. Using bovine leukocytes and nanotechnologies we developed a three-dimensional granuloma model which we designated in vitro granuloma-like structure (IVGLS). We generated stable IVGLS resembling TB granulomas at innate, made of macrophages, or adaptive stages, containing also lymphocytes. Mycobacteria replicated within IVGLS and triggered progression of macrophages towards foamy phenotypes. IVGLS released abundant phagocyte chemoattractants and Th1 cytokines. Magnetic bioprinted bovine granulomas facilitate studying immune responses to mycobacteria, including spatial mapping. Deciphering protective immune responses within IVGLS could contribute to vaccine development for cattle, whereas unveiling resistance mechanisms may help devise novel interventions for human TB.

Session 1.3.2 – Saturday 18 November | 11:06

Theme 7. Immunology of parasite diseases

002 - Improved mucosal response against hemonchosis related to β^A allele of ovine beta-globin gene

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Background: Two β-globin haplotypes have been identified in domestic sheep: A and B. In A haplotype, the β-globin gene cluster includes three highly similar β-globin genes: fetal (β^F), pre-adult (β^C) and adult (β^A); while in B, which bears the adult β^B allele, the cluster underwent a deletion, including the β^C gene. In adult sheep of A haplotype, the expression of β^A can be switched back to β^C during anemia, as a compensatory mechanism, since β^C has an increased oxygen affinity. Therefore, the deletion of β^C globin in sheep of Hb-BB haplotype

leads to decreased tolerance to anemia or hypoxemic conditions. Association between ovine β-globin polymorphisms and resistance against hemonchosis was described, but there are no studies regarding the involved local host responses.
Objectives: Phenotypic parameters and local responses were evaluated in sheep from different β-globin haplotypes naturally infected with *Haemonchus contortus*.

Methods: Morada Nova lambs were monitored at 63, 84, and 105 days of age for faecal egg counts and packed cell volume under natural infection with *H. contortus*. At 210 days of age, lambs of Hb-AA and Hb-BB β-globin haplotypes were euthanized and the fundic region of abomasum was sampled for evaluation of microscopic lesions and relative expression of genes related to immune, mucin, and lectin activities.

Results: Lambs harboring the β^A allele presented an improved resistance against clinical hemonchosis, showing higher PCV during infection. Hb-AA animals presented increased eosinophilia in the abomasum compared to Hb-BB animals, accompanied by higher Th2 profile (*IL4*, *MS4A2*), mucin (*CLCA1*) and lectin activity transcripts (*ASGR1*, *GAL11*), while inflammatory response was increased in Hb-BB animals (*IL1B*, *IL10*).

Conclusion: To our knowledge this is the first study to demonstrate an enhanced local response in the primary site of *H. contortus* infection related to β^A allele of β-globin haplotype.

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Session 1.2.3 – Saturday 18 November | 15:25

007 - Effects of experimental ***Fasciola hepatica*** infection on the long-term immune response of the foot and mouth disease vaccine

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Fasciola hepatica, a worldwide distributed helminth, has a robust immunoregulatory effect in the host, increasing the susceptibility to secondary infections. Foot and mouth disease (FMD) is a highly contagious acute vesicular viral disease effectively controlled by vaccination. Despite the evidence of immunoregulatory effects, the impact of fasciolosis on the immune response induced by FMD vaccination in cattle has never been assessed. Our objective was to evaluate whether the infection by F. hepatica in cattle influences the long-term immunity elicited by the currently used commercial FMD-inactivated vaccines. This experiment used eighteen to twenty-month-old Aberdeen Angus steers negative for F. hepatica. Animals were divided into three groups of 12 (I. Infected; II. Infected-TCZ treatment; III. Control). Animals were infected with 500 metacercariae/animal. After 115 days post-infection (dpi), group II was treated with triclabendazole. Steers were vaccinated twice against FMD virus (FMDV) during the first 6 months of age with Oleolauda bivalent (from Paraguay series 5967700A, formulated with A24/Cruzeiro and O1/Campos strains). Individual serum samples were collected at days 0, 15, 28, 43, 59, 71, 87, 115, 157, and 213 dpi. Indirect ELISAs were used to detect A24/Cruzeiro specific bovine IgG and IgG subtypes. The IgG antibody levels and avidity against FMDV did not show significant differences between all the groups. The commercial vaccine induced higher IgG2 than IgG1 titers in vaccinated animals. Anti-FMDV IgG1 levels significantly decreased in both infected groups at 28 dpi. In addition, the avidity of IgG1 FMDV-specific antibodies at day 28 in the infected group was reduced compared to the control. These results show that F. hepatica infection modified anamnestic responses against FMDV, reducing serum IgG1 titers and avidity. This is the first report of immune-regulation of F.hepatica altering the immune response of FMD vaccines.

049 - Local immune responses to ***Eimeria tenella*** infection in immune chickens – a possible role for interferon-γ induced genes in the inhibition of parasite replication

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Coccidiosis, caused by infection with protozoan parasites of the genus *Eimeria*, is a major problem in poultry husbandry. Infected chickens develop strong, *Eimeria* species-specific protective immunity upon recovery. It is known that Th1-type responses including interferon (IFN)-γ production are central in Eimeria immunity but the effector mechanisms inhibiting parasite replication in the immune host remain largely unknown. The aim of the present study was therefore to identify immune responses induced at the site of parasite replication upon *E. tenella* re-infection of immune chickens. In total 36 SPF-reared chickens were primary infected with *E. tenella* at 18 days of age and re-infected at 53 days of age. Prior to and 1, 2, 3, 4 and 10 days after the second infection, six chickens were sacrificed, caecal tissues collected and RNA was isolated. Messenger RNA was sequenced, and differential expression and gene network analysis was performed using the resulting data. Re-infected chickens had no caecal lesions and faecal oocyst excretion was $3x10^4$-times lower compared to primary infected chickens, confirming that they had achieved protective immunity. Results on mRNA expression in caecal tissue showed 69 differentially expressed chicken genes after the second infection mainly identified 1-2 days after infection. On day 1 67% and on day 2 46% of these were identified as IFN stimulated genes of which 5 were identified as exclusively induced by IFN-γ, and 38 that can be induced by IFN-γ. GO and KEGG analyses of genes expressed days 1 and 2 were consistent with IFN responses and gene network analysis of all expressed genes indicated a response to IFN-γ on day 1. Thus, immune chickens showed a very rapid local response of mainly IFN-stimulated genes revealing an "IFN-γ signature". We hypothesize that IFN-mediated inhibition of parasite replication is an important effector mechanism in protective immunity to *Eimeria* infection.

077 - Dynamics of mucosal and systemic Th2 / Th1 effector T cells in response to tissue migrating ***Ascaris suum*** infections

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The large roundworm *Ascaris suum* is one of the most relevant and frequent soil-transmitted helminths in pigs worldwide. Commonly, early infection and constant reinfection with *A. suum* can be observed. However, initial infection factors that may influence the development of efficient type 2 immune responses during larval migration are poorly understood. Thus, we investigated the local and systemic adaptive immunity along the hepato-tracheal migration route of *Ascaris suum* during primary infection in growing pigs. Pigs were inoculated orally with a single dose of 4000 *A. suum* ova and dissected at selected time points. Immunophenotyping for type 2 and 1 signatures was performed on PBMC and spleen, and compared to local responses in nematode-migration affected tissues (liver, lung, small intestine). Our data indicate that systemic

Th2 levels in PBMC and spleen remain constantly low during larval migration and patent infection. However, we found local and transient Th2 responses in the lung, but not in the liver of infected animals after *A. suum* organ migration. While the initial invasion of the small intestine did not elicit a type 2 response, Th2 cells accumulated locally with the return of larvae and their maturation into pre-adult worms. Interestingly, robust Th1 responses developed both in the circulation and in most of the investigated organs.
Our findings show that in growing pigs a single, experimental infection with *A. suum* does not induce a fulminant systemic type 2 response, but locally and kinetically restricted Th2 immunity. Because Th2 cells are known to orchestrate an efficient anti-parasite response, we propose that the ubiquitous type 1 immune signatures observed in conventionally raised pigs hold back the development of strong type 2 responses. Thus, further research is ongoing to evaluate if the weak type 2 immunity observed might contribute to susceptibility and constant reinfection observed in domestic pigs.

Session 1.2.3 – Saturday 18 November | 16:33

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157 - Equine organoid-derived monolayers as a novel ***in vitro*** model to study host-parasite interactions

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Background: Equine GI nematodes are an increasing problem worldwide due to the development of anthelmintic resistance. To find alternative treatments, a better understanding of the host-parasite interactions and the immune reactions they elicit in the intestine is needed. In that context, organoid cultures are attractive experimental models resembling key features of the intestinal epithelium. However, the large size of nematodes and the closed structure of organoids hinder studies of the natural route of infection. To access the apical surface of the epithelium, we have previously established procedures for transforming equine organoids into monolayers grown in transwells and shown that they are capable of responding to viral and bacterial PAMPs with anti- and pro-inflammatory cytokines.

Objective: To expand the characterization of our equine organoid-derived monolayers, focusing on key epithelial functions in the anti-parasite defence.

Methods: The monolayers were primed with IL-4 and IL-13 before exposed to infectious stage larvae of *Cyathostominae*, *Parascaris univalens* and *Strongylus vulgaris*. Functional effects, including identification of tuft cells and mucus-producing goblet cells, were studied using transcriptional analysis combined with various imaging techniques such as histochemistry, SEM, confocal immunofluorescence microscopy and live-cell microscopy.

Results: Cell-lineage marker analysis, SEM and histological staining of mucins confirmed the formation of heterogeneous monolayers containing both stem cells and differentiated secretory cell lineages. The expression of the goblet cell marker MUC2 and tuft cell marker DCLK1 significantly increased after basolateral stimulation with IL-4/IL-13. In these cytokine-primed monolayers, apical exposure to nematode larvae appeared to promote further MUC2 expression. In addition, live-cell imaging revealed morphological alterations of the monolayers appearing after 48h co-incubation with larvae.

Conclusion: An experimental model representing the equine small intestine is established. The presence of tuft cells and mucus-producing goblet cells responding to external stimuli opens up for more comprehensive studies on equine intestinal defence mechanisms against GI-nematode infections.

Theme 8. Vaccine Development

036 - Immunogenicity of a plant-produced African horse sickness polyvalent vaccine validated in IFNAR mice

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CSIR, Next Generation Health, Pretoria, South Africa

A safe, highly immunogenic polyvalent vaccine to protect against all nine serotypes of African horse sickness virus infection, will revolutionise the AHS vaccine industry globally. We developed plant-produced vaccine candidates that has the potential to protect against all nine serotypes, but equally well can be formulated as mono- and bi-valent formulations for localised outbreaks of specific serotypes. In this study, we formulated a nine-serotype vaccine, administered as two polyvalent (5 µg per serotype) vaccines, and directly compared it to the commercially available AHS live attenuated vaccine. Here we provide compelling data for a nine-serotype vaccine candidate, antigenically distinguishable from one another by LC-MS/MS and ELISA which opens frontiers in AHS vaccines not only in Sub-Saharan Africa but globally. This is the first report of a soluble VP2 fusion protein AHS 9 multi-serotype cocktail VP2 vaccine eliciting the desired titres to all nine serotypes in preclinical studies. We demonstrated elevated Th1 and Th2 titre responses of all 9 serotypes, indicative of protective immunity. The latter paves the way to test safety and immunogenicity of the plant-produced VP2 in horses.

Session 1.3.3 – Saturday 18 November | 15:25

042 - Single-cell RNA-Seq Reveals Multiple Sub-Populations of Bovine Afferent Lymphatic Dendritic Cells Draining the Skin

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Vaccination is a key component in the control of animal pathogens that impact the livestock industry. However, deployable, efficacious vaccines remain unavailable for many prevalent diseases, particularly those requiring cell-mediated immunity for protective efficacy. Dendritic cells trafficking from the site of vaccination are key to determining the downstream adaptive immune response to vaccine antigens. We have utilised a bovine afferent lymphatic cannulation model to collect skin-draining afferent lymphatic dendritic cells (ALDC) in order to perform analysis directly ex vivo in both naïve and vaccinated cattle. We have performed an in-depth characterisation of ALDC in naïve cattle using single cell RNA-Seq and identified multiple subpopulations and trajectories using pseudotime analysis. In addition, we identified a novel cell population that has a similar gene expression profile to monocytes and has functions related to antigen presentation. Further analysis of the transcriptome of ALDC following vaccination could pinpoint targets for enhanced immune induction to increase protective efficacy.

Session 1.3.3 – Saturday 18 November | 15:43

043 - Evaluation of a Live Attenuated Pseudorabies Virus-Vectored Nipah Virus Vaccine

Emily C Ashley[1,2], Rebecca K McLean[1], Walter Fuchs[3], Barbara G Klupp[3], Nazia Thakur[1,4], Dalan Bailey[1], Dirk M Werling[2], Jane C Edwards[1], Thomas C Mettenleiter[5], Simon P Graham[1]

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Pig-to-human transmission was responsible for the first and most severe Nipah virus (NiV) outbreak in Malaysia and Singapore. This outbreak was controlled by culling almost half of all pigs in Malaysia, which caused severe and lasting economic damage to the pig industry. Despite the threat NiV poses to some of the most pig dense regions of the world, no vaccines are currently available. Since NiV outbreaks are rare, there is limited commercial interest in developing a NiV vaccine for pigs. A bivalent vaccine which would protect pigs against infection from a prevalent virus e.g., the pseudorabies virus (PrV), as well as NiV, could provide a financially viable solution. We therefore genetically engineered the live attenuated PrV vaccine strain Bartha K61 to express soluble forms of both the NiV F and G glycoproteins and evaluated its ability to stimulate immune responses against both viruses. NiV-specific T cell responses, determined by IFN-γ ELISpot and flow cytometric assays, were induced by a single immunisation and were enhanced by a booster immunisation. NiV glycoprotein-specific and neutralising antibody (nAb) titres were benchmarked against sera from pigs immunised with previously determined protective vaccine candidates. The recombinant PrV vector induced NiV F- and G-specific antibody titres that were lower than those generated by the other vaccine candidates. However, NiV nAb titres were more comparable, likely reflecting the benefit of inducing antibodies against both NiV glycoproteins. PrV-specific T cell and antibody responses elicited by the recombinant PrV vector were similar to those induced by the parental PrV vaccine strain, strongly suggesting that this would provide comparable protection against PrV. These encouraging initial data support further evaluation of a PrV-vectored NiV vaccine.

Session 1.3.3 – Saturday 18 November | 16:15

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051 - Vaccine immunogenicity in pigs after immunization at different injection sites with liposome-based adjuvants containing specific immunomodulators

Evelína Šťastná[1], Gitte Erbs[1], Kerstin Skovgaard[2], Jeanne Toft Jakobsen[1], Gabriel Kristian Pedersen[1], Gregers Jungersen[1]

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Liposome-based cationic adjuvant formulations (CAFs) are effective adjuvants in mice, non-human primates and humans and are readily amenable for incorporation of immunomodulating molecules to activate e.g., specific pattern recognition receptors and steer immune responses towards a desired outcome. In this study, we used commercial pigs housed under field conditions to investigate the effects of four different CAFs incorporating such distinct immunomodulators: C-type lectin receptor ligands trehalose-6,6'-dibehenate and monomycolyl glycerol in CAF01, toll-like receptor 3 ligand Poly(I:C) in CAF09, or retinoic acid in CAF16 and CAF23. The vaccines were formulated with a recombinant model protein antigen and administered via distinct injection routes. All adjuvants significantly increased antigen-specific IgG and IgA antibodies in serum, compared to non-adjuvanted antigen. Administering the vaccines through intramuscular and intraperitoneal routes induced significantly higher antigen-specific serum antibodies, than the perirectal route. Although the immunizations triggered cell-mediated immunity, no significant differences between the adjuvants or injection sites were detected by intracellular flow cytometry or IFN-γ and IL-17 cytokine-release assays. Gene expression

associated with different T cell subtypes were monitored by microfluidic qPCR, which revealed minor differences only. In the translation from mouse to humans, pigs could play an important role as a large animal model, but our findings suggest that the adjuvant-specific signature of the tested adjuvant immunomodulation does not translate well from mice to pigs. This study provides new insights into immune responses to CAFs in the pig model, and highlights that adjuvant studies should be ideally carried out in the intended species of interest.

Session 1.3.3 – Saturday 18 November | 16:33

065 - In vitro evaluation of cysteine protease and ferritin 2 as vaccine antigens with broad efficacy across avian mites, poultry red mites, tropical fowl mites, and northern fowl mites

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The ubiquitous prevalence of hematophagous avian mites such as poultry red mites (PRMs, Dermanyssus gallinae), tropical fowl mites (TFMs, Ornithonyssus bursa), and northern fowl mites (NFMs, Ornithonyssus sylviarum), become a concern because of their detrimental impact on the production and welfare of poultry. Our research group has been focusing on the anti-PRM vaccine development for controlling PRMs, and several antigen molecules have been identified as effective antigen candidates. The distribution of avian mites is geographically different, and developing a broad efficacy vaccine against avian mites could improve the economic losses of the poultry industry worldwide. For the development of universal vaccines, it is critical to choose the molecules detrimental to physiology and highly conserved among avian mites as ideal vaccine antigens. To assess the potential feasibility as vaccine antigens across mites, we identified cysteine protease (CP) and Ferritin 2 (FER2) which were reported as effective vaccine antigens against PRMs, from TFMs and NFMs, and characterized the potentials of cross-protective vaccine antigens. The deduced amino acid sequences of CP and FER2 of TFMs and NFMs were highly conserved compared with those of PRMs. To assess the protein functions, we separately generated recombinant peptidase domains of CPs (rCP-PDs) and FER 2 (rFER2) from PRMs, TFMs, and NFMs. rCP-PD and rFER2 of each mite exhibited cathepsin L-like enzyme activity and iron-binding ability, respectively. The plasmas of chicken immunized with rCP-PD or rFER2 were cross-reacted with rCP-PD or rFER2 from different mite species. Importantly, the in vitro feeding assays revealed that the survival rates of PRMs fed with each immune plasma against rCP-PD or rFER2 from TFMs or NFMs, in addition to PRMs, were significantly lower than that of the control group in each assay. These data suggest that CP and FER2 could be effective vaccine antigens for developing universal vaccines against avian mites.

Session 3.2.1 – Monday 20 November | 08:30

091 - Aminopeptidase N-mediated transport across the small intestinal epithelium drives gut immunity to oral vaccine antigens in pigs

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Mucosal surfaces serve as primary entry points for pathogens, necessitating the induction of local secretory IgA (SIgA) responses to provide effective protection. While injectable vaccines provide robust systemic immune responses, oral vaccination is the most efficient way to trigger protective gut SIgA responses. Despite this, challenges inherent to the gut tissues impede the development of oral subunit vaccines. On top of vaccine degradation by the harsh environment in the gut and the tolerogenic gut immune responses, another critical hurdle is the poor uptake of vaccine antigens by the gut epithelium. To address this, approaches that facilitate transport across the epithelium to deliver vaccine antigens to the gut immune tissues are much needed. We showed previously that targeting of vaccine antigens to aminopeptidase N enables transport of vaccine antigens across the epithelium and elicits antigen-specific immune responses in pigs. However, the efficacy of this oral vaccine approach to protect against infection remains unclear. Using aminopeptidase N-specific porcinized monoclonal IgA antibodies and FedF, the tip adhesin and a protective antigen of porcine enterotoxigenic E. coli, the causative agent of post-weaning diarrhoea in piglets, we demonstrate that oral delivery of subunit vaccines to the gut immune system via aminopeptidase N triggers secretory IgA responses in pigs, conferring protection against *E. coli* challenge infection. In conclusion, targeted delivery of oral vaccine antigens to aminopeptidase N triggers protective immunity against enteric pathogens in the porcine gut. This delivery technology opens avenues for the development of effective oral vaccination strategies and given the conserved nature of aminopeptidase N, holds promise for translation to other species.

Session 3.2.1 – Monday 20 November | 08:48

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120 - Mannose-chitosan nanoparticle surface adsorbed inactivated influenza virus vaccine elicits cross-protective humoral immunity in pigs

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To mitigate swine influenza A virus (SwIV) infections in swine vaccination is the viable strategy. Lack of induction of mucosal and cellular immunity in the respiratory tract by commercial SwIV vaccines administered intramuscular is responsible for limited cross protection. Our objective is to improve the mucosal and cellular immunity induced by whole inactivated SwIV delivered through mannose-chitosan nanoparticle (mChit-SwIV-NP) by including a STING (stimulator of interferon gene) adjuvant. We developed mChit-SwIV-NP vaccine containing whole inactivated SwIV H1N2 and STING adjuvant ADU-S100, either encapsulated (mChit-SwIV+S100-eNP) or surface adsorbed (mChit-SwIV+S100-sNP). Influenza-free nursery pigs were vaccinated intranasally twice at 3-week interval and challenged with the heterologous pandemic 2009 H1N1 virus (78% HA gene identify). Nasal swabs collected at day post challenge (DPC) 2, 4 and 6, and peripheral blood mononuclear cells (PBMCs), bronchoalveolar lavage fluid (BAL) cells, and tracheobronchial lymph nodes mononuclear cells (TBLN MNCs) isolated at DPC 6 were used for analyses. The infectious virus load was reduced by over 1 log10 in nasal passage of both mChit-SwIV+S100-eNP and mChit-SwIV+S100-sNP vaccinates, with the latter performed relatively better. Consistent with increased specific IgG and sIgA responses with high

avidity and virus neutralizing antibodies in the respiratory tract and serum of mChit-SwIV+S100-sNP vaccinates. While, immunologically, frequency of activated IFNγ+ and IL-17A+ cytotoxic T lymphocytes and T-helper/memory cells in PBMCs and TBLN MNCs, and lymphocytes stimulation index in TBLN MNCs were increased in mChit-SwIV+S100-eNP vaccinates, higher than mChit-SwIV+S100-sNP and commercial SwIV vaccine received groups. In summary, intranasal inoculated mChit-SwIV+S100-sNP vaccine elicited robust cross-reactive antibody responses while mChit-SwIV+S100-eNP vaccine induced robust mucosal and systemic cell-mediated immune responses in vaccinated pigs better than their cohort commercial SwIV vaccine. Suggesting that mannose-chitosan nanoparticle vaccine delivery system has a promise in mitigating SwIV infections and transmission in swine herds.

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Session 3.2.1 – Monday 20 November | 09:06

221 - Single dose new generation heartwater vaccine for smallholder farmers

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Previously, a heartwater pLamp multi epitope DNA vaccine showed protective efficiency when administered three times via the intradermal route (ID) route using the gene gun. However, gene gun immunisation is not practical for use in a rural farm setting. The use of nanoparticles as delivery system for the DNA vaccine could offer a suitable alternative since in this formulation the vaccine can be given as a single dose via IM or subcutaneous (SC) route. In this study, pDNA was encapsulated or adsorbed onto Poly Lactic-co-Glycolic Acid (PLGA) biodegradable nanoparticles. In vitro release profile of pLamp multi epitope DNA vaccine from nanoparticles to simulate a primary and booster vaccination administered as one single vaccine was done. After 24h, a burst release of pDNA was detected in the supernatant. However, by 7-14 days an increased release was observed. A subsequent low release of the remaining pDNA by 21-42 days was evident. Cellular uptake of nanoparticles was studied where a plasmid DNA containing genes for green fluorescent protein (GFP) were adsorbed or encapsulated into polymers. This was used to transfect sheep peripheral blood mononuclear cells (PBMC). The transfected PBMC was analysed by ZOE™ Fluorescent Cell Imager at set time points (24h, 48h, 72h and 96h). The gene expression could be detected within 24 hrs suggesting that the plasmid was taken up by the cells.

Immune responses induced in vitro by the nanoparticle DNA vaccine were evaluated using transcriptome sequencing. Samples were collected from heartwater immune sheep PBMC stimulated with either the nanoparticle alone or with the ME-DNA vaccine adsorbed to the particle at 0h, 6h, 24h, 48h and 72h. Transcription of the plasmid was detected in all the DNAnano samples except at 0h and in none of the nano only samples confirming that the CD4 and CD8 epitopes are transcribed in sheep cells.

Session 3.2.1 – Monday 20 November | 09:24

149 - The ISG15 network is crucial and tightly regulated in the early protection of classical swine fever virus C strain vaccine

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Classical Swine Fever (CSF) is a contagious, severe disease of suids (pigs and wild boar). The infection caused by the classical swine fever virus (CSFV), also referred to as Pestivirus C of the pestivirus genus in the Flaviviridae family. C strain is a highly efficacious live attenuated vaccine that provides rapid and complete protection within 5 days of vaccination. The immunological mechanisms underlying the protection afforded by C strain are poorly understood but precede the adaptive immune response. A previous study had indicated that interferon stimulated genes (ISG) related to ISG15 were at the core of the early immune reaction upon vaccination, supporting the concept that attenuated vaccines provide the immune system with a head start and anti-viral effector proteins confer early innate protection before the onset of an adaptive immune response. We subsequently used an intra-nasal infection and vaccination model to directly compare the C-strain vaccine with the pathogenic Alfort CSFV strain in the tonsil as key site of early virus replication. We could demonstrate that T cell responses might appear within 5 days post vaccination (doi.org/10.3390 /ijms22168795). Using this model we used RNASeq analysis to revisit the role of ISG15 and ISG15-related genes further. Indeed ISG15 related genes dominate the differentially expressed gene list at the start of the C-strain immune reaction – in fact long before vaccine virus occurs in the tonsil. Intriguingly, the core ISG15 gene set identified previously is subsequently down-regulated in vaccinated animals as the adaptive immune response is initiated, while other genes in the ISG15 network are upregulated, but tightly controlled. Further analysis is under way to determine if ISG15 related genes not only play a direct role in virus inhibition, but also prepare the ground for the adaptive immune response arising.

Session 3.2.1 – Monday 20 November | 09:42

Theme 9. Immune-regulation and modulation: Role of mibrobiome

079 - Bovine colostrum supplementation in rabbit diet modulates gene expression of cytokines, gut vascular barrier and red-ox related molecules in the gut wall

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Rabbits play an important role as livestock, pet and laboratory animal. Their sensitivity to bacterial infections presents challenges. Although antibiotics are commonly used to treat these infections, alternative solutions are being sought to prevent the development of antibiotic resistance. Bovine colostrum (BC) has been investigated for its potential in treating and preventing various diseases. The aim of the study was to assess effects of the BC diet supplementation on the rabbit intestinal tract evaluating the gene expression. Thirty female NZW rabbits were randomly divided into three groups according to the diet: control group (commercial feed), 2,5% BC (commercial feed + 2,5% of BC) and 5% BC (commercial feed + 5% of BC). Before weaning the young rabbits were suckled by mothers who had the same diet. The rabbits were weaned and vaccinated (vs Myxomatosis) at 35 days and were fed with the different diets from 35 to 90 days of age. Digestive tracts were removed at slaughter, tissue samples from jejunum, cecum, colon, and lymph node collected for RNA extraction. Blood was also sampled for anti-Myxo Ig titration. Gene expression was evaluated via qRT-PCR. The results indicate that adding 5% BC to the diet results in the upregulation of IL-8 in the jejunum, which not only stimulates inflammation but also facilitates the migration of immune cells. Additionally, the use of BC promotes the expression of genes responsible for the negative regulation of inflammatory responses (TGF-B) and genes which regulate the GVB permeability (CTNNb1). The BC supplementation doesn't seem to influence the efficacy of the vaccination. Furthermore, the inclusion of BC at 5% enhances antioxidant activity in the cecum and colon. These results suggest that BC has significant effects on the rabbit gastrointestinal

tract's inflammatory and antioxidant response, but further research is required to fully understand its histological and physiological impact.

Session 3.1.1 – Monday 20 November | 09:06

Theme 10. Comparative Immunology

013 - Molecular insights into the family of MHC-like molecules (UTs) from marsupials

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Background: The availability of the fully sequenced genome of the *Monodelphis domestica* opossum has enabled the identification and preliminary analysis of a large family of MHC-I-related genes, termed UTs. Unlike the MHC-I chromosomal region, the 17 identified UT genes clustered on chromosome 1 and are only found in marsupials and monotremes. Most of these 17 UT genes exhibit low polymorphism suggesting that they may function as MHC-I-like molecules by presenting non-peptide antigens similarly to MR1 and CD1 molecules in humans.

Objectives: While homology modelling studies suggested that the UT molecules may indeed present antigens, they are yet to be discovered.

Methods: Using a mammalian expression system, the *M. domestica* UT5 molecule was recombinantly expressed and was subsequently crystallized. The UT5 crystals were exposed to X-ray radiation at the Australian synchrotron enabling its three-dimensional crystal structure determination.

Results: Here, we present the crystal structure of the *M. domestica* UT5 to 2.2 Å resolution that revealed the typical overall architecture of a MHC or MHC-like molecule and the presence of a putative endogenously bound ligand within its binding groove that exhibits the molecular features of a lipid-based antigen.

Conclusion: Our findings provide preliminary molecular insights into the evolutionary ancestry of an important class of immune receptor and into the chemical diversity of ligands that can be presented by those molecules.

Session 3.1.2 – Monday 20 November | 10:30

032 - Pathogen recognition by bovine myeloid C-type lectin receptors

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Innate immunity acts as a first line defense mechanism to protect the body from invading pathogens. Myeloid C-type lectin receptors (CLRs) constitute a main class of pattern recognition receptors, mainly recognizing evolutionarily conserved carbohydrate structures of pathogens. While numerous studies have addressed CLRs

in humans and mice, the role of CLRs in large animals is largely unknown. We have previously generated comprehensive CLR-hFc fusion protein libraries of mice, men, and sheep. CLR-hFc fusion proteins are composed of the extracellular domain of the respective CLR, including the carbohydrate-recognition domain, fused to the Fc fragment of human IgG1 molecules. They are useful screening tools to identify CLR/pathogen interactions. This study aims at generating a bovine CLR-hFc fusion protein library to identify CLR interactions with bovine pathogens and to unravel species-specific similarities and differences in pathogen recognition by CLRs. The generated bovine CLR-hFc library consists of Dectin-1, Dectin- 2, MICL, MCL, MGL-1, DC-SIGN, DCIR, MDL-1 and Clec2 and is currently being extended. Several bovine CLR-hFc fusion proteins were tested for functionality using ELISA- and flow cytometry-based binding assays with known ligands for murine or human homologs and yielded positive binding results, e.g., the interaction of bovine Dectin-1 with β-1,3 glucan and heat-killed Candida albicans or bovine DC-SIGN with the S-layer protein of Lactobacillus spp. The bovine CLR-Fc library is used to characterize distinct CLR/pathogen interactions as a first step to analyze the role of CLRs in the bovine immune response. In conclusion, the identification of bovine CLR ligands will provide in-depth insights that may pave the way towards the design of novel adjuvants and immunomodulators in cattle.

Session 3.1.2 – Monday 20 November | 10:48

085 - How do Odocoileus virginianus (white-tailed deer) weather SARS-CoV-2 infection?

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The potential infectivity of SARS-CoV-2 in animals raises a public health and economic concern, particularly the high susceptibility of white-tailed deer (WTD) to SARS-CoV-2. The disparity in the disease outcome between humans and WTD is very intriguing, as the latter are often asymptomatic, subclinical carriers of SARS-CoV-2. To date, no studies have evaluated the innate immune factors responsible for the contrasting SARS-CoV-2-associated disease outcomes in these mammalian species. A comparative transcriptomic analysis in primary respiratory epithelial cells of human (HRECs) and WTD (Deer-RECs) infected with SARS-CoV-2 was assessed throughout 48 hours post inoculation (hpi). Both HRECs and Deer-RECs were susceptible to SARS-COV-2, with significantly ($P < 0.001$) lower virus replication in Deer-RECs. The number of differentially expressed genes (DEG) gradually increased in Deer-RECs but decreased in HRECs throughout the infection. The ingenuity pathway analysis of DEGs further identified that genes commonly altered during SARS-CoV-2 infection mainly belong to cytokine and chemokine response pathways mediated via IL-17 and NF-κB signaling pathways. Inhibition of the NF-κB signaling in the Deer-RECs pathway was predicted as early as 6 hpi. The findings from this study could explain the lack of clinical signs reported in WTD in response to SARS-CoV-2 infection as opposed to the severe clinical outcomes reported in humans.

Session 3.1.2 – Monday 20 November | 11:06

086 - Transcriptional profiling of blood and liver Natural Killer cells reveals the presence of a novel liver Natural Killer cell subset in swine

Leen Hermans, Sofie Denaeghel, Robert J. Jansens, Eric Cox, Bert Devriendt, Herman Favoreel

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The liver is a pivotal organ in the body, yet its cellular composition during steady state remains incompletely understood in most species. Variations in immune cell phenotypes, including liver-resident Natural Killer (NK) cells, further complicate the translation of data between and from animal models to other species, including humans. The porcine liver harbors two distinct NK cell subsets: a CD8α^{high} subset, with a phenotype akin to conventional CD8α^{high} NK cells in peripheral blood, and a CD8αdim subset, which phenotypically closely resembles human liver-resident (lr) NK cells. This suggest that the pig might be an attractive model for studying lrNK cell biology. Here, building further on data obtained from the pig liver cell atlas, we performed bulk RNA-sequencing on FACS-sorted porcine NK cells to compare the transcriptomes of conventional CD8α^{high} NK cells from peripheral blood (cNK cells) as well as CD8α^{high} and CD8α^{dim} NK cells from the liver. Our analysis revealed that highly expressed transcripts in the CD8αdim lrNK cell population mainly include genes associated with the (adaptive) immune response, while transcripts related to cell migration and extravasation were much less abundant compared to cNK cells. Overall, our findings indicate that CD8α^{dim} lrNK cells exhibit an immature and anti-inflammatory phenotype. Interestingly, the CD8α^{high} NK cell population in the liver appears to represent a population with an intermediate phenotype. Indeed, while its transcriptome largely overlaps with that of cNK cells, they also expressed transcripts associated with liver residency, in particular CXCR6. In conclusion, our comprehensive characterization of the transcriptional landscape of porcine liver NK cell subsets lays the foundation to study liver-resident NK cell biology in pigs.

Session 3.1.2 – Monday 20 November | 11:24

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090 - Distinct effector functions mediated by Fc regions of bovine IgG subclasses and their interaction with Fc gamma receptors

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The immune functions and Fc receptor binding profiles of the three cattle IgG subclasses, including complement fixation, NK cell activation and enhancement of phagocytosis, are not fully described. We produced chimeric monoclonal antibodies consisting of a defined variable region linked to the constant regions of bovine IgG1, 2 and 3. These were used to develop functional assays and define receptor binding profiles with His-tagged soluble recombinant bovine FcγRI (CD64), IIA (CD32A), III (CD16) and the bovid-specific receptor Fcγ2R. IgG1 and IgG3, but not IgG2, were found to activate complement-dependent cytotoxicity (CDC). Only IgG1 was capable of activating cattle NK cells to mobilize CD107a after antigen crosslinking, a surrogate assay for antibody-dependent cell cytotoxicity (ADCC). Monocyte-derived macrophages could be triggered by both IgG1 and IgG2 to phagocytose fluorescently labelled antigen-expressing target cells. IgG3 induced only weak antibody-dependent cellular phagocytosis (ADCP). By contrast, monocytes exhibited strong ADCP only when triggered by IgG2. IgG1 bound most strongly to recombinant FcγRs I, IIA and III, with weaker binding by IgG3 and none by IgG2, which bound exclusively to Fcγ2R. Immune complexes containing IgG1, IgG2 and IgG3 bound differentially to leukocyte subsets, with IgG2 binding strongly to neutrophils and monocytes and all subclasses binding platelets. Differential expression of the FcγRs on leukocyte subsets was

demonstrated by surface staining and/or RT-qPCR of sorted cells - Fcγ2R mRNA was expressed in monocytes/macrophages, neutrophils and platelets, potentially explaining their strong interactions with IgG2. These data reveal differences in bovine IgG subclass functionality, which contrast with those of humans, mice and pigs, and should inform future vaccine and therapeutic antibody development.

Session 3.1.2 – Monday 20 November | 11:42

Theme: 11. Zoonoses

041 - Host adaptation of Lagos bat lyssavirus is driven by inhibition of type I interferon

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Bats harbour high-impact viruses such as lyssaviruses. Most of these pathogens have originally been isolated from bats and are zoonotic agents. Despite this association molecular understanding of virus adaptation to bats remains largely unknown. To investigate chiropteran responses to zoonotic viruses we established and comprehensively characterised cell lines of respiratory and brain origin from the Egyptian Rosette Bat (ERB), the natural reservoir host for Lagos Bat Virus (LBV) and Marburg Virus. We observed cell type-specific upregulation of the type I interferon (IFN-I) genes upon stimulation with viral mimetics such as Toll-like or RIG-like agonists. Temperature oscillations, which are unique features of bat physiology, altered kinetics of IFN-I expression. High temperatures boosted IFN-I responses at steady state in ERB cells irrespective of their origin, whereas particularly cells of brain origin were highly responsive to viral mimetics in such conditions. Amongst tested lyssaviruses, LBV solely established successful infection in cells of respiratory origin and viral permissiveness was associated with suppressed expression of IFN-I genes. Lyssavirus phosphoproteins interfered with viral sensing pathway and IFN-I signalling in a host- and virus-specific manner. Our findings provide mechanistical insights into adaptation of lyssaviruses to the chiropteran hosts and enrich knowledge about molecular pathogenesis of rabid disease in reservoirs.

Session 1.2.2 – Saturday 18 November | 10:30

015 - Fasciola hepatica infection in cattle alters the immunity induced by respiratory vaccine

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Fasciola hepatica, a worldwide-distributed liver fluke, is one of the causative agents of fasciolosis, a zoonotic disease that affects livestock and humans. In livestock, fasciolosis causes huge economic losses worldwide, reducing animal fertility, milk production, weight gain and condemnation of livers. In spite of the availability

of drugs, such as triclabendazole (TCZ), for the treatment of fasciolosis, they do not necessarily prevent liver damage or parasite reinfection and can eventually increase parasite resistance. In addition, the parasite induces immunoregulatory mechanisms that evade the host immune response. The aim of this research was to evaluate the immune response induced by the parasite together with the response to respiratory vaccine during parasite chronic infection and before entering to the feedlot facility. We studied the hepatic function, haematological parameters, leukocyte counts in circulation and parasite egg shedding during F. hepatica acute and chronic phases of infection in cattle as well as to determine how these parameters change with TCZ-treatment of chronically infected cattle. Our results show that increased levels of serum aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) were detected in early stages of the experimental infection. Moreover, high circulating eosinophil count and platelets levels were correlated with fluke number in the livers of infected cattle. Interestingly, infected animals showed lower antibody levels induced by the respiratory vaccine when compared to non-infected animals. Interestingly, TCZ-treatment in the chronic phase of infection reduced parasite burden and damage in the liver, and restored antibody titers against Pasteurella multocida and Mannheimia haemolytica induced by the respiratory vaccine during the infection. In conclusion, our work sheds light into the physiopathological mechanisms induced during fluke infection in cattle, revealing the complexity of the host immune response to the infection and the immunoregulatory capacity of the parasite, altering the development of humoral responses induced by vaccination.

Session 1.2.2 – Saturday 18 November | 10:48

108 - Adaptation of Japanese encephalitis virus in pigs can enhance virulence, virus replication, viral shedding and innate immune responses

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Japanese encephalitis virus (JEV) is a Flavivirus infecting wild birds and pigs, associated with the most common viral encephalitis in humans. JEV is endemic in temperate and tropical regions of Asia and Australia, where its main mosquito vector *Culex tritaeniorhynchus* is highly abundant. However, due to climate change, increased travel activity and global trade, the virus may spread to new areas where unlike in Asia, hosts are immunologically naïve. Even though JEV is a mosquito-borne virus, non-vector-borne direct transmission in pigs was observed. Introduction into a naïve population of pigs could therefore result in series of direct transmission, possibly leading to virus adaptation, changes in its virus-host interactions or its potential to become epidemic. To assess the risk, we mimicked a series of direct transmissions in pigs up to passage 10. This virus was then compared to the original stock “P0” in an animal experiment. Five pigs each were infected via the oronasal route. On day 4 post infection, four in contact pigs were added to each stable. Daily samplings for serum and swabs allowed a close monitoring of the virus during infection. The *in vivo* passaged JEV caused a comparable infection, although enhanced virulence, virus replication, viral shedding and stronger innate immune responses could be detected by RT-qPCR, Elisa and transcriptomics. Nevertheless, this prolonged shedding was not associated with enhanced direct transmission. From all sentinels, only one pig got infected, belonging to the “P0” group. To determine what leads to the observed changes, all viruses were sequenced and are currently analyzed. Altogether, this data informs about the capacity of this mosquito-borne Flavivirus to adapt to a single host life-cycle.

Session 1.2.2 – Saturday 18 November | 11:06

Theme 12. Immuno-informatics

025 - Reverse vaccinology approach in designing a multiepitope-based vaccine against ***Babesia*** from rhoptry-associated protein 1 (RAP-1) antigen

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Bovine babesiosis is an important haemoparasitic infection caused by apicomplexan parasites of the genus Babesia. This infection has continuously threatened cattle farmers owing to its devastating effects on production and productivity with serious economic implications. It is also regarded as an emerging zoonosis in humans with fatal outcomes in immunocompromised subjects. Failure to curb the increase of the infection has been attributed to largely ineffective control measures for the disease. These include toxicity and safety concerns associated with residues of chemotherapeutic agents and the limitations of acaricides regarding resistance and environmental contamination. The use of vaccines has been regarded as the most effective and environmentally friendly approach for the control of bovine babesiosis. Currently, vaccinations against bovine babesiosis involve live vaccines and several limitations have been associated with their use. Therefore, searching for safe, reliable, and effective alternative vaccines is imperative. Rhoptry-associated protein (RAP-1), which is immunogenic was used to identify and construct a potential multiepitope vaccine candidate through a reverse vaccinology approach. Antigenic epitopes were identified through the immunoinformatic technique. A multi-epitope vaccine (MEV) comprising 11 CD8+, 17 CD4+, and 3 B-cell epitopes was constructed. The AAY, KK and GPGPG linkers were used to connect the epitopes of the multiepitope construct in addition to the use of Beta defensin 3 as an adjuvant attached at the N-terminal of the vaccine to potentiate the immune response, which was validated through immune simulations. The multiepitope construct was assessed for immunogenicity, antigenicity, structural and physiochemical analysis. The subunit vaccine construct induced and boosted sufficient host cellular and humoral responses, which is important in determining the efficacy of a vaccine. However, validation of these observations through an experimental trial is required.

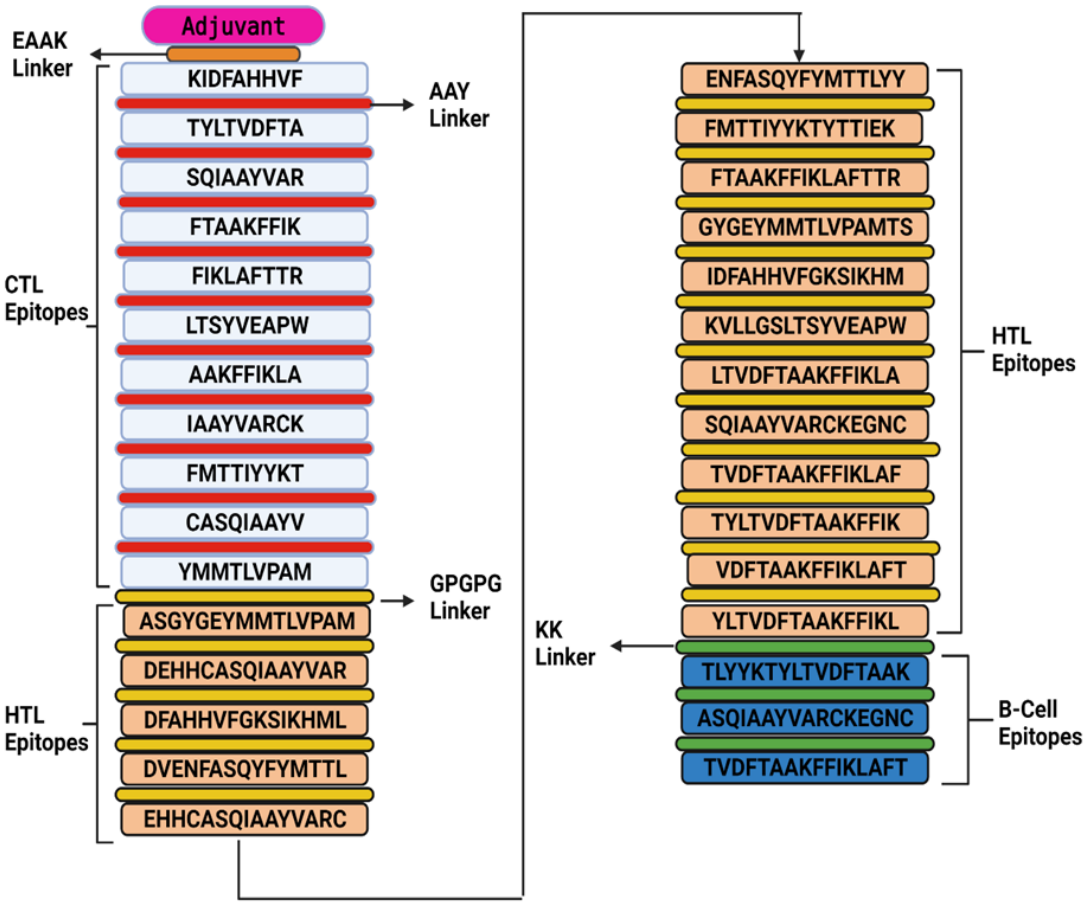

Figure 1. Designed multiepitope vaccine comprising epitopes, linkers, and adjuvant.

Session 1.2.1 – Saturday 18 November | 08:30

058 - Single-cell RNA-seq mapping of chicken peripheral blood leukocytes

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Single-cell transcriptomics provides means to study cell populations at the level of individual cells. In leukocyte biology this approach could potentially aid the identification of subpopulations and functions without the need to develop species specific reagents. The present study aimed to evaluate single-cell RNA-seq as a tool for identification of chicken peripheral blood leukocytes. Blood was collected from 4 clinically healthy hens, leukocytes were purified by Ficoll gradient centrifugation and thrombocytes reduced by immunomagnetic depletion of cells expressing CD41/61. Library preparation and subsequent 3' RNA-seq resulted in transcriptomes from a total of approx. 20000 cells. Bioinformatic analysis of data comprised unsupervised clustering of the cells, and annotation of clusters based on expression profiles. Immunofluorescence phenotyping of the cell preparations used was also performed. Computational analysis identified 31 initial cell clusters and based on expression of defined marker genes 28 cluster were identified as comprising mainly B-cells, T-cells, monocytes, thrombocytes and red blood cells. Of the remaining clusters, two were putatively identified as basophils and eosinophils, and one as proliferating cells of mixed origin. In depth analysis on gene expression profiles within and between the initial cell clusters allowed further identification of cell identity and possible functions for some of them. For example, analysis of the monocyte clusters showed one subcluster comprising heterophils, as well as subclusters of monocytes with differential expression of MHCII and the

mannose receptor. An overall good correlation between mRNA and cell surface phenotypic cell identification was shown. Taken together, we were able to identify and infer functional aspects of both previously well-known as well as novel chicken leukocyte populations although some cell types. e.g., T-cell subtypes proved more challenging to decipher. This methodology definitively has benefits as well as limitations in chicken immunology, e.g., due to the incomplete annotation of the chicken genome.

Session 1.2.1 – Saturday 18 November | 08:48

162 - Immunoinformatics analysis of antigens as candidate vaccines against Eimeria and Toxoplasma for chickens

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Coccidiosis caused by Eimeria species results in huge economic loss and compromise in chickens' welfare in poultry industry globally. Toxoplasma gondii is a zoonosis that affects livestock. Among other things, resistance to control measures has increased the need to search for antigens either to improve or develop new vaccines against coccidiosis and toxoplasmosis in chickens. Eimeria apical membrane antigen 1 (AMA1), immune mapped protein 1 (IMP1) and microneme protein 2 (MIC2) as well as Toxoplasma apical membrane antigen 1 (AMA1), dense granule protein 7 (GRA7) and rhoptry protein-16 (ROP16) protein sequences were retrieved from NCBI. Conserved sequences were used to generate CD8+, CD4+ T-cells and B-cell epitopes following multiple sequence alignment. The epitopes were tested for antigenicity, immunogenicity, conservancy and allergenicity to determine their effectiveness and ability to induce immune responses in the host. Epitopes with antigenic threshold =>0.5 and 100% conserved and immunogenic were selected and further tested for interferon-gamma (IFN-γ) and Interleukin-4 (IL-4) inducer properties. Docking and molecular dynamics simulations were performed on the epitopes. Toxoplasma gondii antigens were fused together with linkers. For Eimeria, the Ramachandran score and Z score of 83.2% and -10.48 (IMP1); 83.6% and -3.48 (MIC2) and 94.46% and -5.21 (AMA1) were obtained. The theoretical pI values were 8.23, 10.26 and 9.85 for IMP1, MIC2 and AMA1 respectively. Instability/aliphatic indexes were 33.40/66.46, 25.18/76.56 and 43.15/70.68 for IMP1, MIC2 and AMA1 respectively. For Toxoplasma gondii, the designed vaccine had 0.6645 antigenicity, 2.89998 immunogenicity score, 73.35 kDa molecular weight, 28.70 instability, 64.10 aliphatic index and -0.363 GRAVY, -151.159 kcal/mol binding affinity. AMA1 is the most suitable antigen for designing vaccine against coccidiosis. Immunoinformatics on Toxoplasma revealed that the Profilin-adjuvanted vaccine is promising, as it predicted the induction of enhanced immune responses through the production of cytokines and antibodies critical in blocking host invasion.

Session 1.2.1 – Saturday 18 November | 09:06

Theme 13. Immunology of wildlife and exotics

081 - Maternal care favors development of distinct immunotypes in a marine mammal

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Maternal care is essential for offspring physiological development and survival in most mammals, including marine mammals. However, how it affects the development of the immune system is poorly understood in wild mammals, but particularly in marine organisms. To unravel the impact of different maternal care and provisioning strategies on immune ontogeny, we captured and monitored wild South American fur seal (Arctocephalus australis) pups and their mothers for 3 years at Guafo Island, Pacific Patagonia. Mothers with shorter foraging trips that spent more time with their pups favored a "less inflammatory" immunotype in their pups characterized by higher levels of interleukin-10 (IL-10), Foxp3+ lymphocytes and basophils. These pups also had a more stable profile of inflammatory cytokines characterized by low within-individual variance in IFN-g and IL-6. On the contrary, a subset of pups that received more sporadic maternal care had an "inflammatory" immunotype characterized by higher levels and variability of IFN-g and IL-6. These group of pups experienced prolonged hookworm infection, struggled to recover from hookworm induced anemia and were more likely to die compared to pups with "less inflammatory" immunotype. By the end of the study, when pups are 12-week-old, these immunotypes also predicted response to an immune challenge (phytohemagglutinin injection). The pups from the "inflammatory" immunotype presented more tissue damage with recruitment of neutrophils relative to the "less inflammatory" immunotype group, which recruited more T-cells in response to the challenge. These results suggest that early maternal provisioning plays an important role in shaping neonatal immunity in wild mammals. These changes have potential implications for the dynamics of infections in juvenile and/or adult individuals with distinct immunotypes.

Session 1.2.2 – Saturday 18 November | 11:24

176 - Elephant Endotheliotropic Herpesvirus (EEHV) Vaccine and First in-Elephant, Proof-of-Concept Trial

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Elephant endotheliotropic herpesviruses are a major threat to juvenile Asian elephants where losses due to EEHV induced haemorrhagic disease (EEHV-HD) remain high. There are no efficacious antiviral therapies or vaccines yet and progress has been hampered by the inability to grow EEHV in vitro. More so, the target cells for EEHV in vivo are unclear and monocytes/macrophages have been proposed as such. Since immunity against herpesviruses particularly relies on cellular responses, we aimed to devise a T cell-inducing vaccine that would elicit both strong cytotoxic and Th1 responses and eliminate any risk of antibody-dependent enhancement of infection through prior vaccination. Accordingly, we applied reverse vaccinology techniques aiming to design a EEHV vaccine that prevents death and severe disease not using external (glycoprotein) antigens. Specifically, we designed a modified vaccinia Ankara (MVA) viral vector as priming vaccine and an adjuvanted protein formulation each containing two antigens as boost, creating a heterologous prime-boost vaccine against EEHV-HD. In a first in elephant proof-of-concept trial, this prototype vaccine was tested for safety (and immunogenicity) in three adult elephants. We used a modified IFNγ release point-of-care, vaccine-specific whole blood stimulation assay as a way to determine T cell responses with RT-qPCR as readout. This was necessary to overcome limitations of sample transport times from the zoo to the laboratory. A complete lack of adverse reactions post-vaccination strongly suggests that this heterologous prime-boost vaccine can be safely used in elephants. Recall IFNγ responses to our candidate antigens were observed against a background of reactions caused by the latent infection that exists in all elephants. Of diagnostic value for the immune reaction were Abs that could be detected against the candidate antigens using Western blot. The results support a further evaluation of this prototype vaccine in young elephants as the target population for vaccination against EEHV-HD.

Session 1.2.2 – Saturday 18 November | 11:42

Theme 14. Immunogenomics and resistance to disease

132 - Inborn errors of immunity in domestic ruminants: lessons learned from two examples

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Background: Inborn errors of immunity predispose animals to infection. Their detection is difficult due to a low frequency and masking by common disease occurrence. Several thousands of dairy cattle and sheep are now routinely SNP genotyped in parallel to records of their performances and life trajectories.

Objectives: Reverse genetics opens new avenues to look for inborn errors that affect the immune system functioning. Using common phenotypes related to disease susceptibility, studies may help detect these errors and establish associated mechanisms.

Methods: Using available phenotypes, two genome-wide association studies were performed to identify candidate genes related to the heightened frequency of mastitis in sheep, and shorter longevity in dairy cattle. Two-point mutations in immune genes were identified and further studied to inform about their effects on immunity.

Results: In dairy sheep, using the milk cell concentration as a proxy for mastitis, the R96C point mutation was identified in SOCS2 with a loss of function. A survey in lactating homozygote ewes showed a high predisposition to infection by Staphylococci, supported by results upon experimental mastitis. In vitro, studies also showed a heightened inflammatory response in gene-edited mice giving definitive proof that the mutation was actually responsible for deregulated innate immunity and susceptibility to infection. In Holstein Friesian cattle, juvenile mortality before calving was found associated with the G307S point mutation in ITGB7, an integrin involved in memory CD4 T cell homing to the digestive tract. Flow cytometry analysis confirmed this defect in homozygote cattle. Moreover, investigations using scRNA-seq and immunohistochemistry showed new alterations of the immune system linked to the loss of integrin beta7, that were disregarded until now.

Conclusion: These two examples emphasize the power of immunogenetics for in-depth analysis of the immune system in species other than humans or mice, for the improvement of animal health and welfare.

Session 3.1.1 – Monday 20 November | 08:30

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160 - Living with only two arms: The TRDC-knockout pigs lacking γδ T cells

Robert kammerer[1], Bjoern Petersen[2], Angele Breithaupt[3], Tung Huy Dau[1], Gregor Meyers[1]

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The tripartite organization of the lymphocytic immune system, consisting of three subsets of lymphocytes: B cells, T cells expressing the αβ T cell receptor (TCR), and T cells expressing γδ TCRs, appears to be fundamental as it evolved independently in jawed and jawless vertebrates. An important variation in the γδ T cell compartments between species is the quantity of circulating γδ T cells, distinguishing γδ T cell low species (e.g., human and mice) from γδ T cell high species (e.g. pig, cow and chicken). While mice deficient for γδ T cells have been established almost 30 years ago γδ T cell deficent mammals representing γδ T cell high species are still missing. Thus, the functional relevance of the different circulating γδ T cell compartments is largly unkown. Knocking-out the porcine TRDC-locus by intracytoplasmic microinjection and somatic cell nuclear transfer resulted in healthy living γδ T cell deficient offspring. Under normal housing conditions no enhanced susceptibility to infections was observed up to 3 years of age. Flow cytometric analysis revealed that TRDC-KO pigs lack completely γδ T cells in peripheral blood mononuclear cells (PBMC) and spleen cells, while heterozygous animals had only a reduced number of γδ T cells. The composition of the remaining leucocyte subpopulations seems to be unaffected by the depletion of γδ T cells. Genome-wide transcriptome analyses in PBMC revealed a pattern of changes reflecting the impairment of known or expected γδ T cell dependent pathways. Histopathology did not reveal developmental abnormalities of secondary lymphoid tissues. Nevertheless, challanging the TRDC-KO pigs with an attenuatted virus revealed that although TRDC-KO pigs stayed healthy they generated a significantly lower neutralizing antibody titer as the syngenic controls, indicating a reduced immunoreactivity.

Session 3.1.1 – Monday 20 November | 08:48

Theme 16. MHC Workshop

020 - Comparative analysis of swine leukocyte antigen (SLA) gene diversity in Göttingen Minipigs

Sabine E. Hammer[1], Tereza Duckova[1], Monica Gociman[1], Sandra Groiss[1], Clara P.S. Pernold[1], Karolin Hacker[2], Lena Kasper[1], Julia Sprung[1], Maria Stadler[1], Andres Eskjær Jensen[3], Peter Vestbjerg[3], Nadine Wenzel[2], Armin Saalmüller[1], Constanca Figueiredo[2]

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Background. Worldwide, pigs represent economically important farm animals, also representing a preferred preclinical large animal model for biomedical studies. The need for swine leukocyte antigen (SLA) typing is increasing with the expanded use of pigs in translational research, infection studies, and for veterinary vaccine design. Göttingen Minipigs (GMP) attract increasing attention as valuable model for pharmacological studies and transplantation research. This study represents a first-time assessment of the SLA gene diversity in Göttingen Minipigs in combination with a comparative metadata analysis with commercial pig lines. As Göttingen Minipigs could harbor private as well as potential novel SLA allele combinations, future research projects would benefit from the characterization of their SLA background.

Methods. In 209 Göttingen Minipigs, SLA class I (*SLA-1*, *SLA-2*, *SLA-3*) and class II (*DRB1*, *DQB1*, *DQA*) genes were characterized by PCR-based low-resolution (Lr) haplotyping. Criteria and nomenclature used for SLA haplotyping were proposed by the ISAG/IUIS-VIC SLA Nomenclature Committee. Haplotypes were assigned based on the comparison with already known breed or farm-specific allele group combinations.

Results. In total, 14 SLA class I and five SLA class II haplotypes were identified in the studied cohort, to manifest in 26 SLA class I but only seven SLA class II genotypes. The most common SLA class I haplotypes Lr-24.0 (SLA-1*15XX or Blank-SLA-3*04:04-SLA-2*06:01~02) and Lr-GMP-3.0 (SLA-1*16:02-SLA-3*03:04-SLA-2*17:01) occurred at frequencies of 23.44 and 18.66%, respectively. For SLA class II, the most prevalent haplotypes Lr-0.21 (DRB1*01XX-DQB1*05XX-DQA*04XX) and Lr-0.03 (DRB1*03:02-DQB1*03:01-DQA*01XX) occurred at frequencies of 38.28 and 30.38%.

Conclusions. The comparative metadata analysis revealed that Göttingen Minipigs only share six SLA class I and two SLA class II haplotypes with commercial pig lines. More importantly, despite the limited number of SLA class I haplotypes, the high genotype diversity being observed necessitates pre-experimental SLA background assessment of Göttingen Minipigs in regenerative medicine and xenograft research.

Session 2.1.2 – Sunday 19 November | 10:30

067 - Role of nonpolymorphic MHC-I and innate-like T cells in resistance and tolerigenic neonatal immunity to mycobacteria

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The growing number of nonclassical MHC-Ib genes and lineages distinct from classical polymorphic MHC-Ia genes identified across jawed vertebrates' genomes raise questions about the importance and function of these genes. To date the amphibian Xenopus is the only species outside mouse and human where a nonpolymorphic MHC-Ib molecules directing the development and function of innate (i)T cells expressing limited TCR repertoire has been characterized. Notably,Xenopus tadpoles provides a unique comparative model for investigating the roles of iT cells in tolerigenic neonatal immunity. While as in mammals, conventional T cells are dominant in adult frogs, tadpoles rely mostly on a few distinct prominent iT cell subsets interacting with cognate nonpolymorphic MHC-Ib (XNC) molecules. We postulate that these iT cells can control inflammatory immune responses against mycobacteria pathogens. Here, we investigated role of the iT cell subset iVα45 (expressing the invariant TCRα rearrangement Vα545-Jα1.14) and its cognate MHC-Ib XNC4 in tolerigenic response to Mycobacterium marinum (Mm) by reverse genetics. Loss-of-function obtained by RNA silencing and CRISPR/Cas9 mutagenesis of either XNC4 or iVα45 T cells dramatically impaired tadpole resistance to Mm. In addition, iVα45 T cell deficiency deregulated immune homeostasis and immune response against Mm. To further examine iT cell response we assessed their proliferation during Mm infection, which revealed to be very limited in tadpole compared to adult frogs and contrasted with the rapid iVa45 T cell recruitment from the spleen to infection sites detected by qPCR and flow cytometry with XNC4-tetramers. Furthermore, tadpole T cells showed a high proliferative rate upon PHA stimulation in vitro but exhibited a lower TCR signaling amplitude than adult frog T cells, which is another hallmark of iT cells. These data suggests that similar to mammalian neonatal T cells, tadpole T cells are functionally distinct from adult counterparts.

Session 2.1.2 – Sunday 19 November | 10:48

129 - A revised view of putative functional histocompatibility genes in the SLA complex

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Background. The pig major histocompatibility complex (MHC), or swine leukocyte antigen (SLA) complex, maps to a large genomic region on chromosome 7. It includes the highly polymorphic series of class I and class II histocompatibility genes involved in peptide presentation and self-recognition. Our aim was to update the annotation of the SLA complex from the Sscrofa11.1 assembly, as an opportunity to enrich our current knowledge on SLA and annotate a whole new haplotype. We found that the SLA complex (2.7Mb from MOG to RING1) is well-assembled, even in the highly duplicated regions that comprise the class I and II gene series. However, the Ensembl or NCBI-based automated annotations of these duplicated genes was not accurate and even misleading.

Methods. We have reannotated the whole SLA genomic region using IsoSeq and RNAseq data from tissues of the Duroc female used for the Sscrofa11.1 assembly.

Results. We have refined the annotation of 27 SLA genes (12 class I genes, 15 class II genes) and have finely characterized their splicing variants. Importantly, we report that SLA-11 initially annotated as a class I pseudogene is a new putative protein coding gene with properties that should lead to its classification as a non-classical class I gene together with SLA-6, -7 and -8.

Conclusions. Our research has clarified the annotation of the SLA genomic region. We confirmed the existence of likely four, not three, functional non-classical class I genes in swine , with specific functions to be elucidated. Our results lead to a revised view of the physical map of the SLA complex by positioning a non-classical class I gene within a cluster of classical class I genes revealing even closer features with the human MHC organization than previously.

Session 2.1.2 – Sunday 19 November | 111:06

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126 - Haplotypic and allelic diversity of non-classical MHC class I in ruminants

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Located approximately 500 kb away from the classical major histocompatibility complex class I (MHC-I) region, the non-classical MHC-I region of cattle contains three MIC genes, three functional non-classical (NC) MHC-I genes, and three NC pseudogenes in the current reference assembly, ARS-UCD1.2. While it is well known that classical MHC-I haplotypes vary in gene content, little is known about the non-classical region, although the variable presence of NC5 has long been noted based on cDNA evidence. We have annotated and compared several complete ruminant non-classical MHC-I haplotypes using existing genome assemblies. All these haplotypes vary considerably from each other in gene content. For example, between zero and six functional MIC loci and between two and six functional NC loci are present in any given haplotype, including putatively functional alleles of Pseudogene 1 and Pseudogene 2 in multiple assemblies. A novel NC locus, proximal to MCCD1, is present in all ruminant haplotypes, but with variable functionality, and appears orthologous to pig SLA-6. This gene content variability was confirmed using MHC-I haplotyped cattle transcriptomes derived from the NCBI Sequence Read Archive. NC5 was found generally associated with both the A14 haplotype and a novel haplotype, while NC3 was absent from all A15 homozygotes and animals with a novel haplotype. In contrast,

NC2 is present and functional in all haplotypes. Relative expression levels of NC2, NC3, and NC5 were found to be on average 2-3x greater than for NC1 and similar to some weakly transcribed classical alleles, while NC4 was expressed on average half as much as NC1. These findings will help inform future studies into the influence of MHC-I variation on immune functions.

Session 2.1.2 – Sunday 19 November | 11:24

Theme 17. Toolkit Workshop

071 - CD38 expression on porcine αβ-T-cell subsets and its role in T-cell activation

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CD38 is a multifunctional transmembrane protein expressed widely by mammalian immune cells, with expression levels varying depending on maturation and activation status. Expression patterns on resting cells have also been reported to vary substantially between species. Following activation, CD38 is upregulated on B- and T-cells in humans and mice, and it appears to play an important role in maintaining T-cell activation via NAD+ depletion and Ca2+ signaling. Investigations into the expression and function of CD38 in the pig have until recently been limited due to the absence of anti-porcine CD38 antibody tools. Here we use a novel generated antibody to quantify cell surface expression of CD38 on porcine immune cell subsets isolated from the blood, spleen, lymph nodes and lung of healthy 6-month-old pigs. Overall, we observed the highest CD38 expression levels on naïve αβ-T-cells, with decreased expression on memory and effector T-cell subsets. Further, plate-bound anti-CD38 mAbs showed a costimulatory capacity on CD3-activated peripheral blood αβ-T-cells, comparable to CD27 or CD28 costimulation. This included upregulation of S6 phosphorylation, proliferation, and cytokine production. Ongoing experiments will assess the effect of CD38 stimulation on the differentiation of FACS-sorted naïve CD4+ T-cells towards mature T-helper cell phenotypes. Our results using novel anti-CD38 mAbs will further elucidate the phenotype and activation of porcine αβ-T-cell populations.

Session 2.1.12 – Sunday 19 November | 08:30

037 - The Immunological Toolbox: Advancing veterinary immunology research through the generation of novel reagents

Inga Dry, Catherine McGuinnes, Emily Charlton, Anna Raper, Zhiguang Wu, Lindsey A Waddell, Jayne Hope

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The UK Immunological Toolbox (www.immunologicaltoolbox.co.uk) is a cross-institute UKRI-BBSRC strategically funded initiative, led collaboratively by the Roslin and Pirbright Institutes. The project aims to reduce barriers to veterinary immunology research by working with the research community to identify gaps within the existing veterinary reagent repertoire.
The services offered by The Roslin Institute core facility include the production of novel reagents, including monoclonal antibodies (mAb), tagged recombinant proteins and ligands, and the development of functional

assays. These advancements will facilitate the understanding of the baseline immune response of a variety of species in addition to their response to disease, pathogens, and vaccines.

Here, we describe an example of novel mAb development enabling studies of the expression of ADGRE1 in cattle. ADGRE1 is a homologue of the widely accepted rodent F4/80/EMR1 monocyte/macrophage marker. The mouse anti-bovine ADGRE1 mAb was used to detect expression on alveolar macrophages by flow cytometry, and on macrophages within a range of tissues by immunohistochemistry. ADGRE1 is uniformly expressed by macrophages in cattle and the mAb is therefore useful for detection of these cells in a range of applications.

Session 2.1.12 – Sunday 19 November | 08:40

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147 - A customizable suite of methods to sequence and annotate cattle antibodies

Kristel Ramirez Valdez[1], Benjamin Nzau[1,2], Daniel Dorey-Robinson[1], Michael Jarman[1], James Nyagwange[3], Liam J Morrison[2], William Mwangi[1], Bryan Charleston[1], Marie Bonnet-Di Placido[1], John A Hammond[1]

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Studying the antibody response to infection or vaccination is key for developing more effective vaccines and therapeutics. Recent developments in high-throughput antibody sequencing technology and immunoinformatics tools facilitate fast and comprehensive analysis of antibody repertoires, with high resolution in any animal species. Here we detail a flexible and customizable suite of methods from flow cytometry, single cell sorting, heavy and light chain PCR amplification to antibody sequencing in cattle. These methods were successfully employed, including adaptation to the 10x Genomics platform, to identify native heavy-light chain pairs. By combining these techniques with the Ig-sequence Multi-species Annotation Tool, we have assembled a powerful toolkit for studying the cattle antibody response with high resolution and precision. Using three workflows, we processed 84, 96 and 8313 cattle B cells from which we sequenced 24, 31, and 4756 antibody heavy-light chain pairs, respectively. Each method has its own advantages and shortcomings in terms of throughput, timeline, specialist equipment, and cost that are each discussed. Moreover, the principles outlined here can be applied to study antibody responses in other mammalian species.

Session 2.1.12 – Sunday 19 November | 08:50

- - END ORAL PRESENTATIONS - -

Poster Presentations

By theme

Theme 1. Tissue Specific Immunity

151 - Effects of chicoric acid on a primary hepatic co-culture of chicken origin treated with viral RNA analogue poly I:C

Máté Mackei[1,2], Patrik Tráj[1], Csilla Sebők[1], Júlia Vörösházi[1], Rege Anna Márton[1,2], Ágnes Kemény[3], Zsuzsanna Neogrády[1], Gábor Mátis[1,2]

[1]Department of Physiology and Biochemistry, University of Veterinary Medicine Budapest, Budapest, Hungary. [2]National Laboratory of Infectious Animal Diseases, Antimicrobial Resistance, Veterinary Public Health and Food Chain Safety, University of Veterinary Medicine Budapest, Budapest, Hungary. [3]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary

Viral diseases impose a significant challenge for the modern poultry sector, as they can lead to reduced production and mortality even in immunized flocks. Since excessive inflammatory response often together with oxidative distress are characteristics of such diseases, systematic mitigation of them may decrease the detrimental consequences. Our main aim was to investigate the use of a viral double-stranded RNA analogue polyinosinic polycytidylic acid (poly I:C), as an effective inducer of inflammation in a chicken primary hepatocyte-non-parenchymal cell co-culture model and to assess the immunomodulatory effects of chicoric acid (CA) and N-acetylcysteine (NAC). Following isolation and differential centrifugation, cell cultures were incubated with 50 µg/ml poly I:C alone or combined with 10 (C1) and 100 (C2) µg/ml CA or 100 (N1) and 200 µg/ml (N2) NAC. Metabolic activity, lactate dehydrogenase (LDH) activity, interleukin-6 (IL-6), -8 (IL-8), -10 (IL-10), interferon alpha (IFNα) and gamma (IFNγ) and macrophage colony-stimulating factor (M-CSF) concentrations were measured from the medium. Concentration of malondialdehyde (MDA), and caspase-3 were monitored in the cell lysates. According to our results, increased LDH activity was reduced in poly I:C treated cells by CA, moreoverC2 treatment was also able to restore cellular metabolic activity. Concentrations of pro-inflammatory cytokines were reduced by most of CA and NAC treatment compared to the poly I:C-induced cytokine spike. Decrease in MDA concentration was observed following the lower dose treatment of CA compared to the group exposed to poly I:C alone. Our model proved to be effective in monitoring the cytotoxic response induced by viral inflammation. CA showed a dose-dependent cytoprotective and anti-inflammatory potency. In conclusion, based on our in vitro experiments, CA may be a promising candidate to mitigate the damage caused by double-stranded RNA viruses and thus improve the health and productivity of poultry.

Poster Session 2 – Sunday 19 November

Theme 2. Immuno-diagnostic

034 - Revisiting the correlation of c-Kit mutation status and treatment decisions in canine mast cell tumors

Sabine E. Hammer[1], Andrea Fuchs-Baumgartinger[1], Christof A. Bertram[1], Nicole Luckschander-Zeller[2], Ilse Schwendenwein[1], Barbara C. Rütgen[1]

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Background: Mast cell tumors (MCTs) are the most frequent skin tumors in dogs, with an incidence of 16-21% of all tumors. Mutations in the proto-oncogene *c-Kit*, encoding the transmembrane stem cell factor receptor on mast cells, induce constitutive receptor activation. Up to 50% of canine MCTs exhibit internal tandem duplications (ITDs) in either exon 8 or 11 promoting cell growth and survival. We aimed to establish an indication for the treatment with tyrosine kinase inhibitors (TKIs), based on the *c-Kit* mutation status of the canine MCT patients.

Methods: In 50 dogs, tumor stage was histopathological confirmed by Kiupel and Patnaik grading systems. The mutation status of *c-Kit* exons 8, 9, 11, 13, 14 and 17 was investigated by isolating genomic DNA from remnant diagnostic material and subsequent sequence comparisons to healthy and malignant reference material.

Results: For exon 8, one ITD was found, and only 10 patients showed ITDs in exon 11, both leading to constitutive activation of c-KIT protein. The mutation in exon 8 and three of the ITDs in exon 11 correlated with low-grade (Kiupel)/ grade II (Patnaik) tumor stage. In contrast, seven of the MCT dogs carrying an ITD-mutated exon 11 were staged high-grade (Kiupel)/ grade III (Patnaik). In one patient, the low-grade (Kiupel)/ grade II (Patnaik) tumor stage did correlate with an amino acid exchange V563D (T>A^{1688}) in one of the Ig-like domains coding exon 9. In human, this mutation drives gastrointestinal stromal tumors, whereas in canine mast cell tumors the Valine to Aspartic Acid exchange is responsible for TKI resistance.

Conclusions: For *c-Kit* mutation analysis, a time- and cost-efficient work routine was established. The low ITD frequency in exons 8 and 11 together with the absence of other know mutations suggest that the *c-Kit* mutation status alone is not sufficient to make treatment decisions.

Poster Session 2 – Sunday 19 November

076 - Comparison of the Bionote NSP Ab ELISA and PrioCheck FMDV NS ELISA

Khomotso Confidence Moabelo[1], Angelika Loots[1], Sven Parsons[2], Melvyn Quan[1]

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Foot and mouth disease (FMD) is a highly contagious viral disease that causes devastating economic losses due to decreased productivity, trade restrictions and control measures. Accurate and timely diagnostics are crucial for effective disease management, including early detection, control, and prevention. The detection of antibodies against non-structural proteins (NSP) of the FMD virus (FMDV) is one of the best tools to determine natural FMDV infection and can be used to differentiate infected from vaccinated animals. The purpose of the study was to evaluate the diagnostic agreement between the Bionote NSP Ab ELISA and PrioCheck FMDV NS ELISA. Four hundred and one serum samples were obtained from the Agricultural Research Council-Onderstepoort Veterinary Institute, Transboundary Animal Disease (ARC-OVR, TAD) Biobank. Results indicated that 284 (70.8%) samples were negative on both the Bionote and PrioCheck ELISAs, 62 (15.5%) serum samples tested positive on both ELISAs, and 11 (2.7%) and 44 (10.9%) samples tested positive on the Bionote and PrioCheck ELISAs, respectively. Cohen's Kappa coefficient for the two tests was calculated to be 0.61, depicting moderate to substantial agreement between the two ELISAs. Based on these findings, it was concluded that both the Bionote and PrioCheck ELISAs demonstrate effectiveness in detecting FMDV NSPs. It also suggests a degree of confidence in their ability to identify positive samples. However, the disparity in the number of samples testing positive on each test suggests that there may be variations in sensitivity or specificity between the two ELISAs. Further investigations are warranted to identify the factors contributing to these differences and to determine the optimal circumstances in which each test should be used. Overall, the evaluation provides valuable insights into the performance of the Bionote NSP Ab ELISA and PrioCheck FMDV NS ELISA and highlights their potential applications in FMD diagnostics.

Poster Session 2 – Sunday 19 November

134 - Leukocyte dynamics in vaccinated and unvaccinated animals following dual challenge with Bovine Viral Diarrhea Virus-Mannheimia haemolytica model to compare vaccine efficacy in calves

Stephanie Perkins-Oines[1], Sonja VanHolland[1], Karim Abdelsalam[1], Norah Bate[2], Joy Drach[2], Christopher CL Chase[3,1]

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Bovine respiratory disease continues to be a major threat to calf health. The study objective was to determine whether vaccination would affect differential leukocyte changes following a dual challenge with Bovine Viral Diarrhea Virus (BVDV) and M. haemolytica. In this study colostrum-fed calves were vaccinated at ~30 days of age with either a placebo (CON), a licensed combination parenteral vaccine that contained 5 viruses including BVDV type 1 and type 2, and M. hemolytica toxoid or concurrently with intranasal temperature-sensitive (TS) with 3 respiratory viruses and a parenteral vaccine containing modified live BVDV type 1 and type 2 and M. haemolytica toxoid. The calves were challenged ~5 months after vaccination with BVDV 1b and then 7 days later with M. haemolytica. Blood was collected for blood leukocyte differential (BLD) count using the QScout-BLD chute side system on days 0, 3-10 and 12. In this study, the major innate pro-inflammatory cells, segmented neutrophils, monocytes and band neutrophils and the major anti-inflammatory cell, the eosinophil, were good indicators of the vaccine status of the animal. Control animals had lower absolute segmented neutrophils and lower monocytes following BVDV infection compared to vaccinates while % differential was unaffected. Following M. haemolytica challenge, there was an absolute increase in segmented neutrophils, monocytes and band neutrophils and a decrease in eosinophils in the controls compared to vaccine groups. The comparison with QScout-BLD indicated that lung lesions were seen in animals with higher segmented and band neutrophils and monocytes and lower numbers of lymphocytes and eosinophils. This is consistent with our understanding that higher number of proinflammatory cells and the lower number of anti-inflammatory cells are associated with lung damage. Although peripheral blood is not the lung inflammatory cell milieu, the BLD provided surrogate indicators of protection from respiratory disease in this model system.

Poster Session 2 – Sunday 19 November

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155 - Development of a reverse transcription quantitative real-time PCR assay for the evaluation of a multivalent inactivated vaccine

Zinathi Dlamkile[1], Luis Neves[1,2], Darshana Morar-Leather[1], Junita Liebenberg[3], Helena Steyn[3], Erika Faber[3], Alri Pretorius[3]

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Heartwater is a tick-borne disease caused by *Ehrlichia ruminantium* in the sub-Saharan Africa region and Caribbean islands. *Amblyomma* genus ticks are responsible for transmitting the pathogen and in South Africa, the pathogen is transmitted by *A. hebraeum*. In southern Africa, the disease is controlled by regular use of acaricides and immunization. However, the vaccine offers limited cross-protection against some virulent *E. ruminantium* isolates. Effective vaccines are a requirement to conquer the fight against heartwater. Developed vaccines must be tested for their ability to elicit protective immunity against heartwater. In this study, a reverse transcription quantitative real-time PCR (RT qPCR) assay for the evaluation of a multivalent inactivated vaccine

was developed. Sheep were vaccinated with an inactivated multivalent heartwater vaccine and blood was collected at different time points. Housekeeping genes were selected and validated for relative quantification of mRNA levels of immune markers. The selection and validation methods were by a statistical algorithm that include Ct mean, CV of RPKM values, and CT values, and the ΔCt method, and the genes were ranked using RefFinder. Immune markers differentially expressed between surviving animals and treated animals were identified using RNA-seq data from a previous study. Relative changes in gene expression of immune markers were measured by RT qPCR. The best housekeeping genes were SDHA, RPL22, YWHAZ, and GAPDH and the appropriate immune markers were CD41–ITGA2B, CD156A-ADAM8, CXCR5, CD8B, CD5, GZMB-LIKE, IFN-γ and TGFB1. The developed RT qPCR assay revealed that the immune markers were differentially expressed at all time points. Although an increase in expression of the immune markers in all vaccinated animals at least one of the two-time points post-vaccination, it does not predict survival. Therefore, an increase in the expression of the immune markers post-vaccination cannot be used to indicate protection. The RT qPCR assay can be validated.

Poster Session 2 – Sunday 19 November

172 - Assessment of serum IgG-avidity and IgG1 titres as predictors of heterologous protection against foot-and-mouth disease in vaccinated cattle

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Foot-and-mouth disease (FMD) caused by FMD virus (FMDV) is a disease of cloven-hoofed livestock. FMD can be controlled using inactivated vaccines which protect against the vaccine's homologous virus strains; but cross-protection is usually feeble, even within strains of the same serotype. Homologous protection afforded by FMD vaccines correlates strongly with virus-neutralising antibody titres (VNT), however, animals with undetectable VNTs are protected against heterologous strains. Challenge studies are then used to assess the efficacy of vaccine antigens with field viruses. These trials are time-consuming, expensive and against animal welfare. Serology has been proposed as an alternative to challenge trials, provided a good correlation with protection can be demonstrated. Our laboratory developed indirect ELISAs that use purified 146S whole viral particles to assess FMDV-specific IgG avidity and subtypes, proposed to be more convenient for this purpose. In this study, we analysed if IgG-avidity ELISA and IgG1-IgG2 titres correlate with cross-protection. Sera from 17 non-protected and 23 protected animals from three different PD50 A22/Iraq challenge trials were tested (1). Animals were vaccinated 21 days before the challenge with monovalent vaccines containing A/Iran96 or A/Iran 99 strains. VNTs were below detection levels in 26 animals, so no further analysis was possible. No differences in IgG2 titres were found between protected and non-protected animals. However, IgG1 titres as well as avidity indexes of IgG against A22/Iraq were significantly higher in protected compared to non-protected animals ($p<0.05$). ROC curves were built to establish cut-off values and conditional testing was applied to increase sensitivity. In these conditions, the accuracy of the prediction was 86% and concordance with protection was 0.7 (Kappa value). These results confirm the feasibility of using whole-particle indirect ELISAs that detect IgG1 and IgG avidity to assess cross-protection in FMDV-vaccinated cattle.

(1) K.E. Brehm et al. Vaccine (2008) 26, 1681—1687

Poster Session 2 – Sunday 19 November

175 - Comparative analysis of four diagnostic methods to detect Fasciola species in naturally infected slaughter dairy cattle

Zuko Mpisana[1], Ishmael Festus Jaja[1], Charles Byaruhanga[2], Sunday Ochonu Ochai[2], Sunday Charles Olaogun[3], Veronique Dermauw[4], Pierre Dorny[4], Munyaradzi Christopher Marufu[2]

[1]University of Fort Hare, Department of Livestock and Pasture Science, Alice, South Africa. [2]University of Pretoria , Department of Veterinary Tropical Diseases, Pretoria, South Africa. [3]University of Ibadan, Department of Veterinary Medicine, Ibadan, Nigeria. [4]Institute of Tropical Medicine, Department of Biomedical Sciences, Antwerp, Belgium

The diagnosis of bovine tuberculosis (bTB) faces challenges pertaining to test characteristics of existing methods and logistical considerations. It follows that for the control of bTB, development of novel, accurate and easy-to-use diagnostic methods is essential. Recently, microRNAs (miRs) have emerged as a highly promising novel class of diagnostic biomarkers in various other infectious and non-infectious diseases, including mycobacterial infections such as (human) tuberculosis. The aim of this pilot study was to provide proof of principle that miRs may be differentially expressed in animals infected with M. bovis or vaccinated with Mycobacterium bovis (BGC) versus healthy animals, laying the foundation for the of use of miRs as diagnostic biomarkers for bTB with DIVA potential. To this end, the global microRNAomes of cattle either vaccinated with M. bovis BCG (n=3; week 8) and challenged with M. bovis with no visible lesions at post-mortem ("protected"; n=3; week 17), or unvaccinated animals (n=3; week 8) subsequently challenged with M. bovis with visible lesions ("unprotected/infected"; n=3; week 17), were characterized using RNA-sequencing. Briefly, total RNA was extracted from peripheral blood mononuclear cells and small-RNA libraries prepared for Illumina sequencing (Novaseq 50bp, SE). An average of 13.4M reads were generated per sample, 97.3% of which had a Q-score of >30. Alignment of the reads to the bovine reference genome bosTau9 (ARS-UCD 1.2) using miRDeep2 and miRBase v22 revealed the presence of ±472 (range: 406-524) known and ±20 (range: 10-28) novel miRs across all samples. Using DESeq2, 2 miRs were found to be significantly differentially expressed (log2fold change≥|1|; adjusted p-value ≤ 0.05) in protected versus control animals: bta-miR-34a was downregulated, and bta-miR-29b upregulated. Our data suggest that these miRs could be indicative of M. bovis infection or vaccination and therefore implies their potential as diagnostic biomarkers for bTB in cattle.

Poster Session 2 – Sunday 19 November

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193 - The MicroRNAome of Cattle Infected with Mycobacterium bovis: Towards the Characterization of Potential Novel Biomarkers

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[1]Utrecht University, Department of Biomolecular Health Sciences, Utrecht, Netherlands. [2]Utrecht University, Department of Population Health Sciences, Utrecht, Netherlands

The diagnosis of bovine tuberculosis (bTB) faces challenges pertaining to test characteristics of existing methods and logistical considerations. It follows that for the control of bTB, development of novel, accurate and easy-to-use diagnostic methods is essential. Recently, microRNAs (miRs) have emerged as a highly promising novel class of diagnostic biomarkers in various other infectious and non-infectious diseases, including mycobacterial infections such as (human) tuberculosis. The aim of this pilot study was to provide proof of principle that miRs may be differentially expressed in animals infected with M. bovis or vaccinated with Mycobacterium bovis (BGC) versus healthy animals, laying the foundation for the of use of miRs as diagnostic biomarkers for bTB with DIVA potential. To this end, the global microRNAomes of cattle either vaccinated with

M. bovis BCG (n=3; week 8) and challenged with M. bovis with no visible lesions at post-mortem ("protected"; n=3; week 17), or unvaccinated animals (n=3; week 8) subsequently challenged with M. bovis with visible lesions ("unprotected/infected"; n=3; week 17), were characterized using RNA-sequencing. Briefly, total RNA was extracted from peripheral blood mononuclear cells and small-RNA libraries prepared for Illumina sequencing (Novaseq 50bp, SE). An average of 13.4M reads were generated per sample, 97.3% of which had a Q-score of >30. Alignment of the reads to the bovine reference genome bosTau9 (ARS-UCD 1.2) using miRDeep2 and miRBase v22 revealed the presence of ±472 (range: 406-524) known and ±20 (range: 10-28) novel miRs across all samples. Using DESeq2, 2 miRs were found to be significantly differentially expressed (log2fold change≥|1|; adjusted p-value ≤ 0.05) in protected versus control animals: bta-miR-34a was downregulated, and bta-miR-29b upregulated. Our data suggest that these miRs could be indicative of M. bovis infection or vaccination and therefore implies their potential as diagnostic biomarkers for bTB in cattle.

Poster Session 2 – Sunday 19 November

Theme 3. Innate Immunity

080 - Different immune response against Gram positives and Gram negative mammary infection revealed by two dairy cow breeds

Federica Riva[1], Joel Filipe[1], Giulio Curone[1], Alessia Inglesi[1], Susanna Draghi[1], Valerio Bronzo[1], Claudia Pollera[1], Renata Piccinini[1], Massimo Amadori[2], Daniele Vigo[1]

[1]Università degli Studi di Milano, Milan, Italy. [2]Rete Nazionale Immunologi Veterinari, Brescia, Italy

Bovine mastitis represents one of the major concerns in dairy industry due to high production losses, therapy-associated and early cull costs, with relevant public health implications. Recent studies focused on mastitis resistance, but the mechanisms of susceptibility to intra-mammary infections still need to be clarified. It is well known that Gram-positive and negative pathogens induce different immune responses in the mammary gland of dairy cows. In this scenario, we focused on the bacterial killing activity of milk and colostrum. Accordingly, we defined a new protocol, based on flow cytometry analysis, to investigate bacterial killing. Freshly cultured, log phase E. coli and S. aureus strains, isolated from clinical cases of bovine mastitis, were incubated under the same conditions with acellular milk and colostrum sampled at different time points from two groups of cows (allegedly mastitis resistant and susceptible breed, respectively), and analyzed for vitality by flow cytometry following incorporation of vital dyes. N-acetyl-β-D-glucosaminidase (NAGase) activity was also investigated in milk and colostrum samples. We observed that colostrum and milk bacterial killing activity is mainly expressed against S. aureus compared to E. coli. Bacterial killing is seemingly correlated with NAGase activity. Both killing of S. aureus and NAGase activity are negatively correlated with the day of lactation. Interestingly, bacterial killing and NAGase activity were greater in colostrum and milk samples from reputedly mastitis-resistant cows. Our study confirms that different pathogens induce different immune responses in the mammary gland of dairy cows. Our findings are conducive to a better understanding of pathogenesis and immunoregulation during mastitis. This could help define in the future new diagnostic markers and therapeutic protocols against specific pathogens.

Poster Session 1 – Saturday 18 November

082 - Characterization of polarization states of canine monocyte derived macrophages

Qingkang Lyu[1], Edwin Veldhuizen[1], Irene Ludwig[1], Victor P. M. G. Rutten[1], Willem van Eden[1], Alice J.A.M. Sijts[1], Femke Broere[1,2]

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Macrophages can reversibly polarize into multiple functional subsets depending on their micro-environment. Identification and understanding the functionality of these subsets is relevant for the study of immune-related diseases. However, knowledge about canine macrophage polarization is still in its infancy. In this study, we polarized canine monocytes using GM-CSF/IFN-γ and LPS towards M1 macrophages or M-CSF and IL-4 towards M2 macrophages and compared them to undifferentiated M0 monocytes. Polarized M1 and M2 macrophages were thoroughly characterized for morphology, surface marker features, gene profiles and functional properties. Our results showed that canine M1-polarized macrophages obtained a characteristic large, roundish, or amoeboid shape, while M2-polarized macrophages were smaller and adopted an elongated spindle-like morphology. Phenotypically, all macrophage subsets expressed the pan-macrophage markers CD14 and CD11b. M1-polarized macrophages expressed increased levels of CD40, CD80 CD86 and MHC II, while a significant increase in the expression levels of CD206, CD209, and CD163 was observed in M2-polarized macrophages. RNAseq of the three macrophage subsets showed distinct gene expression profiles, which are closely associated with immune responsiveness, cell differentiation and phagocytosis. However, the complexity of the gene expression patterns makes it difficult to assign clear new polarization markers. Functionally, M0-monocytes, and M1- and M2- like subsets of canine macrophages can all phagocytose latex beads. M2-polarized macrophages exhibited the strongest phagocytic capacity compared to non-polarized M0- and M1-polarized cells. Taken together, this study showed that canine M1 and M2-like macrophages have distinct features largely in parallel to those of well-studied species, such as human, mouse and pig. These findings enable future use of monocyte derived polarized macrophages particularly in studies of immune related diseases in dogs.

Poster Session 1 – Saturday 18 November

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113 - Immunological Response to Increased Intestinal permeability on Endurance Exercise Horses

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Endurance riding is a popular equestrian sport. These horses perform for extremely long distances in high demand of intense aerobic metabolism. These leads to physiologic changes resulting in splanchnic hypoperfusion, loss of intestinal barrier function, and subsequent immunological response to components of the intestinal lumen [e.g., lipopolysaccharide (LPS)] that have crossed the barrier. LPS binding protein (LPSBP), is a critical acute phase protein with a role in innate immune response to increased intestinal permeability (IIP). Immunological changes in response to endurance exercise are important due to the exquisite sensitivity to endotoxin (LPS) in horses. Changes in LPSBP in response to intense prolonged exercise has not been evaluated in horses. The objective of this study was to document changes in LPSBP and immunologic response to IIP to endurance exercise in horses. We hypothesized that there will be evidence of IIP in this population. 60 horses participating in an endurance ride. Blood samples were collected for plasma separation prior to the beginning of the ride (Pre), middle of the ride (Mid), immediately after the ride (Post), and 24 hours after the end of the ride(24h). The concentrations of LPSBP were evaluated via enzyme-linked immunosorbent assay at the different time points. Data were analyzed with a repeated measures ANOVA with Dunnett's test for correction of multiplicity of comparisons. A P-value of less than 0.05 was considerate significant.

Results indicated that there was an increase in LPSBP over time during the ride. There was no significant change from Pre to Mid. There was a significant increase from Pre to Post (P=0.02) Importantly, the highest concentrations were captured 24h (P=0.0002). Concluding, our results confirmed LPSBP increases marginally immediately post-exercise but the magnitude increase is far greatest 24 hours after exercise, as would be expected with IIP and activation of the innate immune response.

Poster Session 1 – Saturday 18 November

123 - The immunomodulatory action of the antimicrobial peptide IDR-1002 in a hepatic cell culture model of chicken origin

Csilla Sebők[1], Patrik Tráj[1], Máté Mackei[1], Ágnes Kemény[2], Zsuzsanna Neogrády[1], Gábor Mátis[1]

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The inadequate application of antibiotics both in human and veterinary medicine has contributed to the global spread of antibiotic resistance. Hence, it is highly important to find alternative candidates for combating bacterial infections and alleviating the triggered inflammatory response in livestock farming. For instance, Innate Defense Regulator (IDR) peptides are synthetic antimicrobial peptides, which could be promising tools due to their potent immunomodulatory activity. In the present study, the immunomodulatory effect of IDR-1002 was investigated in a primary hepatocyte – non-parenchymal cell co-culture of chicken origin under lipoteichoic acid (LTA)-induced inflammation. Cells were exposed to 50 µg/ml LTA and 10, 30, 90 µg/ml IDR-1002 alone and in combination for 24 h. Thereafter, the metabolic activity of the cells was assessed by CCK-8 assay, while the concentrations of several pro- and anti-inflammatory mediators were determined in culture media by Luminex magpix technology. Based on the results obtained, IDR-1002 did not affect the metabolic activity of the cells, confirming the absence of cytotoxicity. IDR-1002 application had a remarkable impact on the inflammatory homeostasis of the cultured liver cells. The administration of the peptide alone decreased both pro-inflammatory IL-6 and anti-inflammatory IL-10 levels, while increased the concentration of M-CSF and RANTES, influencing macrophage differentiation to anti- or pro-inflammatory direction, respectively. This suggests that macrophages exhibited properties characteristic for both pro- and anti-inflammatory types. LTA exposure stimulated the cellular CXCLi2, IL-6 and IFN-γ release; however, this excessive pro-inflammatory cytokine release was alleviated by concomitant IDR-1002 application, confirming its anti-inflammatory action in LTA-evoked inflammation. It can be concluded that IDR-1002 exerted a highly complex effect on the cellular immune response and thus may be a promising candidate for the treatment of pathologies associated with inflammation of bacterial origin in poultry farming.

Poster Session 1 – Saturday 18 November

133 - Which factors modulate the bovine whole blood response to LPS: Nature vs Nurture?

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Background: In high-yielding dairy cattle, the transition period around calving is characterized by a high predisposition to infection and inflammation. The mechanisms involved are still poorly understood. Identifying predisposing factors is essential for setting up preventive measures.

Objectives: The purpose was to assess innate immune responsiveness about susceptibility to infection using experimental conditions that can be implemented in the field, do not interfere with the sale of products, and are respectful of animal welfare.

Methods: LPS is frequently used to assess immune response, at least for innate immunity. A protocol using whole blood stimulation with LPS in an all-in-one step and multiplex profiling of cytokine production was developed. A group of 110 SNP-genotyped dairy cows with high-precision phenotype records in a single herd was used to investigate the influence of genetic and environmental traits on this specific response. Validation was done in a multicentric study with 400 dairy cows.

Results: Cytokine secretion with LPS was significantly different from the control condition for most cytokines, with IL-1β, IFNγ, and TNFα being the cytokines that contributed most to differentiating the two conditions. Cows gathered into three clusters according to the response intensity. The most influential factor in the LPS response was lactation rank with a parallel increase of most cytokine concentrations. Milk yield and metabolic traits correlated significantly to the production of particular cytokines such as IL-6 and IL-17 in the LPS condition, while the estimated breeding value for milk cell concentration was negatively correlated with several other cytokines in the control.

Conclusion: Although this new protocol for assessing immune response contributes to identifying factors linked to health-related events in dairy cattle, other kinds of stimulus would be useful for a wide description of the immune responsiveness to exploit this approach for genetic selection or health improvement purposes.

Poster Session 1 – Saturday 18 November

150 - In vitro screening of immunostimulatory ligands for induction of trained immunity in porcine monocyte/macrophages

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Compiling evidence has suggested that, similar to acquired immunity, the cells of innate immune system also memorize the initial encounter to endogenous or exogenous insults which equip them with a greater ability to fight against secondary homogenous or heterogenous stimuli. So, the focus of the present study has been dedicated to find the immunomodulatory compounds that can induce trained immunity in porcine monocyte/macrophages. Using an in-vitro approach of trained immunity already established in human monocyte/macrophages, we screened a large array of pathogen and damage associated molecular patterns (PAMPs and DAMPs) to find the ligands with the potential to induce trained immunity by measuring the levels of pro-inflammatory cytokines as the first hallmark of trained immunity. Different b-glucans including (scleroglucan, postulant, curdlan, sizofiran, b-glucan peptide, laminarin, b-glucan from Saccharomyces cerevisiae, zymosan, b-glucan from Euglena gracilis), Pam3CSK, LPS, Poly I:C, flagellin, IFNγ, uric acid, resiquimod and guardiquimod failed to induce innate immune memory in porcine monocyte/macrophages. However, priming of porcine monocytes with muramyl di-peptide (MDP), a NOD2 ligand, induced enhanced levels of pro-inflammatory cytokines like TNF, IL-1β and IL-6 upon second stimulation with heterogenous stimuli including LPS, Poly I:C and zymosan. Priming of monocytes in the presence of 2-deoxy glucose (inhibitor of glycolysis), wortmannin (Akt inhibitor) and resveratrol (Sirtuin1 histone deacetylase activator) inhibited the training effects of MDP suggesting the possible role for metabolic and epigenetic reprogramming as the underlying mechanism. Further investigations are underway to address the impact of MDP on epigenetic and metabolic landscape of porcine monocyte/macrophages using transcriptomics, Seahorse XF technology and ATAC-seq.

Poster Session 1 – Saturday 18 November

152 - Harnessing trained immunity to enhance resistance of piglets against infections

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Many infections of gastrointestinal and respiratory tract target the farm animals during the weaning. Confronting the multi-factorial post-weaning complications requires multiple actions such as hygienic and husbandry measures, feed additives and boosting the immune system. The latter can be achieved in part via the long-term, non-specific protective effects offered by the concept of trained immunity. Therefore, the ultimate goal of the present study is to examine the impact of innate immune memory on improving the resistance of piglets against post weaning diarrhoea (PWD). To meet this goal, we screened a large array of pathogen and damage associated molecular patterns using an in-vitro model of trained immunity already established in human monocyte/macrophages. Relied on the in-vitroresults, groups of six piglets were trained with PBS, Seppic® adjuvant (Adj), Adj+ all trans retinoic acid (Adj-ATRA), Adj+ß-glucan (Adj-BG), Adj+ATRA+ ß-glucan (Adj-ATRA-BG) via IM route at the age of 14 days. To mimic the in-vitro model of trained immunity, piglets then received a single dose of LPS adjuvanted Mycoplasma hyopneumoniae vaccine (HyogenÒ) one day after weaning (age of 29 days). We assessed the PBMCs transcriptome, antibody response, pro-inflammatory cytokines and clinical scores to determine the immune training effects. Regarding the clinical score (body weight, temperature, faecal score) and pro-inflammatory cytokines we did not observe any differences between groups. Antibody response peaked at day 21 post vaccination without showing any distinctions between our study groups. Assessing the blood transcriptomics modules using GSEA, we observed almost the same enrichment pattern for PBS and Adj-ATRA-BG. The other groups showed a decreased innate immune response upon vaccination compare to PBS group similar to what is normally observed in innate immune tolerance. In conclusion, none of our studied compounds were able to induce trained immunity in piglets.

Poster Session 1 – Saturday 18 November

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177 - Canine amniotic fluid at birth: From a discarded sample to a potential diagnostic of neonatal maturity and health

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The definition of new reliable markers for neonatal maturity evaluation is crucial in canine clinical practice. Amniotic fluid had been considered waste material until the latest studies reported amniocentesis as a reliable and safe procedure, even in the canine species. In our study, amniotic fluid collected at birth from ten dogs undergoing elective Caesarean sections at term was analysed to discover new potential indices of canine neonatal maturity and health. Some of the mothers were treated with Alizin and all of them were vaccinated against Parvovirosis, Hepatitis and Distemper. In particular, the content of lecithin, sphingomyelin, surfactant protein A, cortisol, pentraxin 3, anti-Parvo anti-hepatitis anti-distemper IgG were investigated in amniotic fluid. Maternal serum SP-A, cortisol, anti-parvo anti-hepatitis anti-distemper IgG were also measured. All amniotic parameters were detectable in canine amniotic fluid. Interestingly, the concentrations of different amniotic parameters correlated with each other. Lecithin was positively correlated with sphingomyelin, maternal SP-A, and the ratio of amniotic and maternal cortisol. Amniotic SP-A was inversely correlated to maternal SP-A, lecithin, and lecithin-sphingomyelin ratio. A positive correlation was also recorded between amniotic and maternal cortisol. Considering that all puppies were born alive and mature, these data could provide a

potential range of expected amniotic values in full-term new-born dogs. Since gestational age was positively correlated with both maternal and amniotic cortisol and amniotic PTX3, amniotic fluid seems to be an attractive, innovative, and minimally invasive matrix with potential diagnostic and prognostic utility for the investigation of canine maturity. Moreover, in the amniotic fluid of puppies from mothers treated with Alizin we observed a significant increase of PTX3 expression, cortisol and sphingomyelin concentration and increased title of anti-hepatitis and anti-distemper IgG. In the amniotic fluid of puppies from vaccine high responder mothers we identified significant higher titles of anti-parvo IgG.

Poster Session 1 – Saturday 18 November

187 - Heterogeneity of Phenotypic and Functional Changes to Porcine Monocyte-Derived Macrophages Triggered by Diverse Polarizing Factors In Vitro

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Swine is one of the most relevant large animal biomedical model, due to many anatomical, physiological, and immunological similarities with humans. However, porcine macrophage polarization has not been extensively analyzed in this specie. Therefore, we investigated porcine monocyte-derived macrophages (moMΦ) triggered by either IFN-γ + LPS (classical activation) or by diverse "M2-related" polarizing factors: IL-4, IL-10, TGF-β, and dexamethasone. IFN-γ and LPS polarized moMΦ toward a proinflammatory phenotype, although a significant IL-1Ra response was observed. Exposure to IL-4, IL-10, TGF-β, and dexamethasone gave rise to four distinct phenotypes, all antithetic to IFN-γ and LPS. Some peculiarities were observed: IL-4 and IL-10 both enhanced expression of IL-18, and none of the "M2-related" stimuli induced IL-10 expression. Exposures to TGF-β and dexamethasone were characterized by enhanced levels of TGF-β2, whereas stimulation with dexamethasone, but not TGF-β2, triggered CD163 upregulation and induction of CCL23. Macrophages stimulated with IL-10, TGF-β, or dexamethasone presented decreased abilities to release proinflammatory cytokines in response to TLR2 or TLR3 ligands: IL-10 showed a powerful inhibitory activity for CXCL8 and TNF release, whereas TGF-β provided a strong inhibitory signal for IL-6 production. While our results emphasized porcine macrophage plasticity broadly comparable to human and murine macrophages, they also highlighted some peculiarities in this species.

Poster Session 1 – Saturday 18 November

189 - PMN support Brucella dispersal with reduced immune recognition

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Neutrophils (PMNs) are the first line of defense against bacteria entering the body. However, it has been shown that *Brucella* induce low PMN activation and survive within these leukocytes by resisting their microbicidal mechanisms. *Brucella* also induce the premature cell death of PMNs, which release chemokines and express "eat me" signals. These PMNs are then phagocyted by mononuclear cells where *Brucella* replicate. This evidence suggests that PMNs may behave as vehicles protecting *Brucella* from immune recognition, favoring their dispersion to the target organs. To test this hypothesis, we analyzed the course of infection in mice

intraperitoneally infected with *B. abortus* alone (Ba) or with *Brucella*-infected PMNs (Ba-PMN). We evaluated bacterial loads, histopathological analyzes, cytokine production in serum, anti-*Brucella* antibody titers, and hematological parameters. We observed that mice infected with Ba-PMN had lower bacterial loads in the spleen and bone marrow at seven days of post-infection compared to Ba. The bacterial load then became equivalent at 30 days post-infection. The pathological index demonstrated a similar trend to the bacterial load. Comparably, Ba-PMN infected mice showed less IFN-gamma and IL-6 at the beginning of the infection than the Ba group but with similar concentrations at the end of the experiment. Ba-PMN infected mice also showed fewer anti-*Brucella* antibody titers at day 30 than the Ba group. No significant differences were observed between infected groups in the hematological values at 30 days. Despite both groups reaching similar bacterial loads by day 30, the bacterial loads and the immunological parameter were lower at the beginning of the infection in the Ba-PMN group. We conclude that the course of infection in the Ba-PMN group was stealthier than in the Ba group. Despite the slower course of *Brucella* infection in the Ba-PMN, PMNs supported their dispersion to a similar extent but with reduced immune recognition.

Poster Session 1 – Saturday 18 November

Theme 4. Adaptive Immunity

022 - Unravelling signals important for early chicken B-cell development

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Understanding the development of the humoral immune system in livestock species, including poultry, is essential for improving animal health and welfare, reducing disease transmission, and advancing our understanding of infectious diseases. Embryonic B-cell development is crucial in establishing a strong, lifelong humoral immune response, which relies on B-cells to produce antibodies that aid in protecting against various infectious diseases. Diversification of B-cell receptors (BCRs) results in efficient humoral immune responses during lifetime by generating a wide range of antibody specificities against multiple diseases. Thus, understanding embryonic B-cell development is of utmost importance. In birds, B-cell precursors are first located in the embryonic spleen. From embryonic day 10 (ED10) onwards, these immature B-cells start to migrate through the bloodstream and colonize the bursa of Fabricius, a specialized avian immune organ responsible for B-cell development. There they undergo further development to become mature naïve B-cells, which includes diversificatio0n of their B-cell receptor. However, signals that lead to the exclusive immigration of chicken B-cells into the bursa and guide them to the bursa remain largely unknown. It has been shown that successful rearrangement and expression of surface immunoglobulin are required for the emigration out of the bursa. However, it is not essential for precursor B-cells to migrate to the bursa. The chemokine CXCL12 was shown to play a significant role in precursor B-cell immigration into the bursal anlage via CXCR4, but this strongly depends on BCR expression. Thus, more signals steering the B-cell migration remain to be identified. To analyze their transcriptome, we isolated chicken B-cells from the spleen, blood, and bursa at ED12, ED14, and ED16. Data analysis of differentially expressed genes between organs and time points will reveal novel mechanisms guiding precursor B-cells migration. Thereby, we will gain more insight into early avian B-cell development.

Poster Session 1 – Saturday 18 November

075 - Immune checkpoint blockade for cancer immunotherapy in dogs: updated results from a clinical study using anti-PD-L1 antibody (c4G12)

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Immune checkpoint inhibitors, such as anti-programmed cell death 1 (PD-1) and anti-PD-ligand 1 (PD-L1) antibodies, represents a promising immunotherapy for various cancer types in humans. Previously, we have demonstrated that PD-1 is highly expressed on canine tumor-infiltrating lymphocytes and that PD-L1 overexpression is a common feature of various malignancies in dogs. These observations suggest that the reversal of immunosuppressive tumor microenvironment by immune checkpoint blockade is also beneficial in canine cancers. A canine chimeric anti-PD-L1 antibody, c4G12, has been developed and tested for its safety and clinical efficacy in a pilot clinical study, demonstrating the tolerability and antitumor activity in a small population of dogs. Here we report an update of the clinical study using c4G12 in dogs with pulmonary metastatic oral malignant melanoma (OMM; *n* = 29). Treatment-related adverse events (TRAEs) of any grade were observed in 15 dogs (51.7%), including grade 3 TRAEs in four dogs (13.8%). Of particular interest, one dog developed grade 3 pneumonitis (3.4%). Tumor response was evident by diagnostic imaging in five of 29 dogs (17.2%). Survival was significantly longer in the c4G12 treatment group, when compared to a historical control group (*n* = 15) that were treated with standard therapies in the same veterinary hospital (143 days vs. 54 days). Our results show that anti-PD-L1 antibody (c4G12) is well-tolerated and effective against pulmonary metastatic OMM. Further clinical studies are ongoing to reveal its clinical benefit in different clinical settings.

Poster Session 1 – Saturday 18 November

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083 - Cellular and Humoral immune responsiveness to inactivated Leptospira interrogans in dogs vaccinated with a tetravalent Leptospira vaccine

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Although vaccination is used to protect dogs against leptospirosis, a zoonosis caused by pathogenic Leptospira, little is known about adaptive immune responses induced by Leptospira vaccines. In the present study, antibody and T cell mediated responses were assessed in dogs before and 2 weeks after yearly vaccination with a commercial tetravalent Leptospira vaccine. Our results demonstrate a significant increase in Leptospira-specific IgG titers after immunization of dogs. Furthermore, CD4+ and CD8+ lymphocytes proliferated after vaccination following incubation with the inactivated L. interrogans serogroup Canicola or Australis vaccine strains. Increased frequencies of central memory CD4+ T cells as well as central and effector memory CD8+ T cells defined by expression of CD45RA and CD62L markers, and increased expression of the activation marker CD25 were found on T cells after vaccination. Increased secretion of CXCL-10 and IFN-γ in the supernatant of Leptospira stimulated PBMC was found after vaccination as well. Together, our results provide more in-depth knowledge on the involvement of IgG- and T cell responsiveness, potentially contributing to protection, upon yearly revaccination with the tetravalent Leptospira vaccine.

Poster Session 1 – Saturday 18 November

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111 - Immune biome of Bovine Colostrum

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Background: Immunoglobulins are key mechanisms by which the host targets not just pathogens but also members of the commensal microbiota. However, the rational for immunoglobulins targeting commensal microorganisms is not entirely clear.

Objectives: We hypothesise that immunoglobulins targeting bacteriophage may play a role in shaping the neonatal gut microbiome's composition.

Methods: To test this hypothesis, we profiled bacteriophage communities from colostrum samples from Holstein Friesian cows using targeted shotgun metagenomics, on whole colostrum as well as immunoglobulin-enriched samples, IgG- and IgA-associated bacteriophage.

Results: Beta diversity analysis suggests that these communities were highly animal specific, despite shared housing and feed. Bacteriophage communities displayed a high predominance of lactococcal phage in colostrum prior to first feeding. In addition to the assay used to separate immunoglobulin-bound from free bacteriophage, scanning electron microscopy images confirmed the presence of IgA-associated bacteriophage, confirming the phenomenon of immune system recognition of bacteriophage. Interestingly, in a subset of paired faecal and colostrum samples undergoing 16S rRNA sequencing demonstrated no overlap in bacteriome communities, suggesting that maternal faecal microbiota do not play an important role in the initial colonisation of the neonatal microbiome.

Conclusion: Our results suggest that the distinct colostrum biome may be important in initial colonisation and establishment of immune tolerance.

Poster Session 1 – Saturday 18 November

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144 - The influence of the germline antibody loci on the pig and warthog antibody repertoire

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In the last several years, several highly contiguous genome assemblies have been generated for pigs and related species. These include the Sscrofa11.1 reference assembly and USMARCv1.0, as well as yet-to-be released assemblies for the warthog and the inbred Babraham pig. We have annotated the antibody heavy chain (IGH) and light chain (IGK and IGL) genes within these assemblies. Despite being contiguous in Sscrofa11.1, the IGK locus was confirmed by BAC sequencing to be mis-assembled in all Sus scrofa assemblies, this includes approximately 300 kb of unplaced sequence containing dozens of IGKV gene segments. All three loci were also found to vary considerably between assemblies in their V gene segment content, indicating likely substantial haplotypic variation. While variable across the entire V region in IGH and IGK, gene content variation was largely confined to the IGLC gene segments and to the IGLC-proximal gene segments belonging to the IGLV3 subfamily within the IGL locus. In addition to having only a single utilized IGHD and single IGHJ gene segment, Sus scrofa is further constrained to a single functional IGHV subfamily. The warthog is similar, however, the complete IGH locus in the warthog assembly contains only two putatively functional IGHV gene segments that differ by only 12 bp (~96 % identity). To better understand how this surprisingly limited germline

diversity relates to the expressed repertoire, we sequenced the IgM antibody repertoire from PBMCs derived from the same animal used in the genome assembly. While expected variation was found across complementarity determining region 3, a considerable amount of variation was also found across the rest of the V region and concentrated in predicted cytidine deamination hotspots. There was also no evidence of extensive somatic gene conversion. Together this suggests that warthogs rely on somatic hypermutation to diversify their pre-immune repertoire.

Poster Session 1 – Saturday 18 November

154 - Increased antigenic broadness in the adaptive humoral immunity elicited by different Foot and Mouth Disease vaccine regimes against heterologous viral strains

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Foot-and-mouth disease (FMD) is one of the WOAH notifiable diseases due to its potential for rapid spread among domestic and wildlife biungulates, with severe economic impacts. Its etiological agent, the FMD virus (FMDV) is a small non-enveloped positive-stranded RNA virus with high antigenic variability in its capsid proteins. FMDV vaccines may prevent clinical FMD after infection with homologous strains, however its protective ability is usually modest against heterologous strains. This study analysed if increased heterologous virus neutralization may be achieved among serotype O FMDV strains, when immunization is performed using heterotypic FMDV isolates, particularly after revaccination, increasing of the antigenic payload or including additional FMDV strains in the vaccine formulation. Naïve cattle were immunized with a set of seven FMDV vaccines with different strain composition and antigenic payload. Each experimental group (n=3) received three immunizations at 0, 28 and 56 days post-primary vaccination to test the effect of revaccination and serum samples were taken at different time-points up to 70 days after initial vaccination. Immune sera were tested by a virus-neutralizing test (VNT) against six serotype O FMDV strains from the same topotype as the vaccinal strain (O1/Campos) and from other topotypes from South-East Asia. After the second revaccination, the heterologous neutralizing capacity was improved particularly in vaccines containing the O1/Campos strain, as monovalent (high payload), bivalent o trivalent formulations against most of FMDV strains tested. High payload O1/Campos monovalent vaccines reached significant neutralizing titres against heterologous strains faster than lower payload formulations. Interestingly, two strains isolated during the outbreak (O Ecu 46/10 and O Ecu 56/10) presented very different responses, indicating that even subtle differences in capsid proteins, may impact in the ability of the immune sera to neutralize the infective FMDV.

Poster Session 1 – Saturday 18 November

159 - Characterization of novel memory marker on porcine T cells

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Memory formation is an important feature of the adaptive immune system and crucial for the control of many infectious diseases. CD4 T cells can be divided into naïve, central memory (TCM), effector memory (TEM), and terminal effector (TEMRA) subsets. Recently, a novel monoclonal antibody (mAb) was identified (clone 6C5 2H4), that specifically stains memory subsets of porcine TCR-αβ T cells and is completely absent on naïve T cells. On $CD4^+$ T cells, this marker is expressed on a subset of $CD8\alpha^+CD27^-$ TEM but is also absent on

CD8α^{+}CD27^{+} TCM. Likewise, on CD8β^{+} cytolytic T cells the novel memory marker is expressed on CD11^{high}CD27$^{-/dim}$ CD8β late and early TEM and absent on CD11^{low}CD27^{+} TCM. We observed that after PMA/Ionomycin stimulation only a minor subset of the newly identified memory subset was able to produce cytokines like IFN-γ, TNF-α, and IL-17A. In upcoming experiments, memory subsets identified by the 6C5 2H4 mAbs will be investigated in the setting of viral infection to further elucidate the role of this marker in a model of antigen-specific memory. Up to date, the cognate antigen recognized by the novel mAb is not identified but a screening on a porcine cDNA library is currently ongoing. Results obtained so far as well as future work on this new memory subset will help to improve understanding antigen-specific immune response in the pig.

Poster Session 1 – Saturday 18 November

185 - Evaluation of Haematological Biomarkers of Immune Response in Chickens following *in vivo* Administration of Inactivated Avian Influenza H5 Vaccine

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Background: Highly Pathogenic Avian Influenza outbreaks is a great concern for public health and the survival of the poultry industry. This necessitates the adoption of control measures such as biosecurity and or vaccination.

Objective: This study was designed to assess the hematological changes in chickens administered with inactivated avian influenza H5 vaccine via the intramuscular (IM) and subcutaneous routes (SC).

Methods: A total of ninety (90) one-day-old ISA Brown chickens (30 each) obtained from three major commercial hatcheries (A, B and C) were used for this study. The chicks from each hatchery were divided into 3 groups (1, 2 and 3) and administered different doses (0.2 mL, 0.5 mL and 0.7 mL) of the AIV H5 vaccine via either IM or SC routes at 14 and 28 days of age. Blood was collected from each chick at 14, 21, 28, 35, and 42 days of age, and evaluated for hematological changes.

Results: Our findings revealed no significant differences ($P > 0.05$) in the packed cell volume (PCV) of all the chicks from the 3 different hatcheries administered all doses of the vaccine via IM route. There was significant ($P < 0.05$) difference at 14 days in PCV of chicks from hatcheries B and C administered 0.5 mL of the vaccine via SC route. The total leukocyte count (TLC) was statistically significant between chicks from hatcheries A and B at 14 days using IM but showed no significant ($P > 0.05$) differences using SC. Heterophil and lymphocyte counts showed significant ($P < 0.05$) differences in all chicks. The heterophil-lymphocyte ratio (H/L) was significantly different ($P < 0.05$) in all chicks at 35 days (IM) and 14 days (SC).

Conclusion: This study demonstrated enhanced hematological changes in ISA Brown chicks administered with different doses of inactivated avian influenza H5 vaccine via the intramuscular and subcutaneous routes.

Theme 5. Immunology of viral diseases

016 - Essential oils able to completely inhibit the avian coronavirus replication

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Background: Avian coronavirus (AvCov) genome is well known to be highly prone to undergo mutations, leading to changes on amino acid sequences of the major structural AvCov proteins, especially in the spike (S) glycoprotein. Since the S protein is involved on the virus attachment to the host receptors and the most important neutralizing epitopes, mutations in this gene could result in antigenic changes and emergence of AvCov variants. The constant arising of new AvCov variants is pointed out as the main cause of worldwide vaccine failures in the field. Therefore, we aimed to explore alternative methods of controlling AvCov that could potentially be effective across all AvCov strains. Since essential oils (EO) from botanicals has been reported for a number of viruses, they were here investigated.

Method: Syzgium aromaticum and Cymbopogon martin were tested under different concentrations for toxicity evaluation on embryonated chicken eggs. The EOs dilutions of 1% and 0.1% were incubated during 30 minutes with H120 strain of AvCov, and inoculated in embryonated chicken eggs Specific Pathogen Free. A fixed viral titter of 101.54 EID50 were administered per egg. The inoculated eggs were daily observed during seven days, and the chorion allantoid liquid (CA) was harvest for absolute quantification of AvCov RNA by RT-qPCR. The experiment was performed three times.

Results: The 1% dilution of both EOs was able to completely inhibit the viral replication, presenting no embryo lesions or RNA virus detection in the CA, while the 0.1% dilution presented a partial to absent inhibition of virus replication, presenting embryo lesions and RNA virus detection on the CA.

Conclusion: This is the first report to demonstrate antiviral activity of Syzgium aromaticum and Cymbopogon martin EOs. Further experiments are required to prove same effect in chickens.

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Poster Session 1 – Saturday 18 November

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056 - Different macrophage polarization patterns induced by porcine reproductive and respiratory syndrome viruses (PRRSV)

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Diverse genetic variation is a distinctive characteristic of porcine reproductive and respiratory syndrome virus (PRRSV). Infections with different PRRSV strains result in varied clinical outcomes and disease severity. The highly pathogenic (HP)-PRRSV induces extensive inflammatory responses, while the PRRSV-1 (EU) and PRRSV-2 (US) viruses are usually associated with immunosuppression and secondary complications. The ability of PRRSV to target macrophages plays a crucial role in its immunopathogenesis. Macrophages can exhibit two distinct polarization states, known as M1 and M2, which allow them to facilitate different pattern of immunological cascades. M1 macrophages are involved in inflammatory responses, whereas M2 macrophages contribute to immunosuppressive functions. We hypothesized that different strains of PRRSV might induce

different patterns of macrophage polarization. To validate the hypothesis, porcine monocyte-derived macrophages (MDM) were *in vitro* cultured with PRRSV-1 (strain DV, MLV), PRRSV-2 (strain 01NP1, Thai isolate), or HP-PRRSV (strain 10PL01, Thai isolate) for 48 hours, at 37 °C, 5% CO2. The MDM cultured with polarizing cytokines or mock-infected MARC-145 cell lysate (mock) were included as controls. Following the incubation period, the cells were collected and subjected to various immunological analyses. The results demonstrated that HP-PRRSV selectively induced M1 macrophage, as indicated by upregulations of pSTAT, TLR4, iNOS, IL-1β and IL-6 expression. Conversely, PRRSV-1 and PRRSV-2 selectively induced M2 macrophage through upregulations of pIRF4 and ARG and IL-10 expressions. The varied immunological, and the subsequent clinical, outcomes following infections with different PRRSV strains appeared to correlate with the pattern of macrophage polarization. Our findings highlight the significance of macrophage polarization in PRRSV immunopathogenesis and provide insights for future intervention strategies.

Poster Session 1 – Saturday 18 November

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110 - Predict and Protect against PRRSV (PreProPRRSV): Combining PRRSV forecasting technology with vaccine efficacy prediction to prevent PRRSV outbreak

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The high mutation rate of PRRSV represents a big challenge and raises two important questions for swine producers: Which PRRSV strain will hit my farm next? And which vaccine can best protect my herd against it? Currently no technology can adequately answer those questions. To overcome this issue, we have combined two state-of-the-art technologies – PRRSV forecasting and heterologous vaccine efficacy prediction. These technologies will create the first proactive PRRSV mitigation system: Predict and Protect against PRRSV (PreProPRRSV). Thereby, for the first time, PRRSV outbreak mitigation will become proactive! The PreProPRRSV system integrates two components:

1. Establishment of PRRSV Forecasting Technology. This forecasting methodology uses computer-based prediction algorithms based on surveillance data relevant to predict PRRSV spread – both intrinsic (e.g., variation of pathogen strains) and extrinsic (e.g., landscape) variables, pig transporting, and farm locations. This technology can precisely predict the spread of PRRSV strains in North Carolina (NC).
2. Establishment of a Vaccine Efficacy Prediction System. This system consists of an immune biobank (cells + serum) from pigs which received different PRRSV vaccinations. This biobank is established at the North Carolina State Veterinary College and will enable to determine which vaccine induces the strongest immune response to an approaching NC PRRSV strain.

This interdisciplinary project combines computer-algorithm-based forecasting with translational immunology to enable precision animal management for PRRSV in North Carolina: It will determine the most effective vaccine before the emerging PRRSV strain arrives at a production site. The PreProPRRSV system aims to enhance pig health and production by decreasing the impact of PRRS with a proactive outbreak mitigation approach.

Poster Session 1 – Saturday 18 November

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145 - Host immune response to FMDV SAT 1, 2 and 3 infections: Defining mechanisms of persistent infection in cattle.

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Foot-and-mouth disease (FMD) is a cloven-hoofed notable livestock disease with both economic and social negative impact. The disease is caused by FMD virus (FMDV), a Picornaviridae member of the genus Aphthovirus. This virus exists through seven immunologically distinct serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia 1). There is inadequacy in the understanding mucosal responses and all immune mechanisms of protection during acute and persistent infections in cattle. Thus, the aim of this project is to determine immune mechanisms underlying protection against FMDV SAT 1, 2 and 3 infections throughout distinct phases of infection in cattle. Cattle will be infected with FMDV SAT 1, 2 and 3 virus using intranasopharyngeal (INP) inoculation system. ELISPOT will be used for detection of FMDV-specific antibody-secreting B cells. Multiplex staining panel developed to differentiate cattle B and T cells into putative functionally distinct subsets will be used to characterize B and T cell response to FMDV infection. Furthermore, immune transcriptome regulation will be determined to characterize global immune response to FMDV using the RNA-sequencing technology. Understanding of transmission mechanism underlining persistent FMDV infection and activation of innate and adaptive immune response in livestock will assist in disease control strategies. Furthermore, immune transcriptome data will also assist in development of next-generation vaccines and antiviral products against FMDV.

Poster Session 3 – Monday 20 November

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188 - Host immune response to FMDV SAT 1, 2 and 3 infections: Defining mechanisms of persistent infection in cattle

Mpendulo F Mtimunye[1], Selaelo I Tshilwane[1], Kgomotso P Sibeko-Matjila[1], Tshifhiwa Nefefe[2]

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Foot-and-mouth disease (FMD) is a cloven-hoofed notable livestock disease with both economic and social negative impact. The disease is caused by FMD virus (FMDV), a Picornaviridae member of the genus Aphthovirus. This virus exists through seven immunologically distinct serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia 1). There is inadequacy in the understanding mucosal responses and all immune mechanisms of protection during acute and persistent infections in cattle. Thus, the aim of this project is to determine immune mechanisms underlying protection against FMDV SAT 1, 2 and 3 infections throughout distinct phases of infection in cattle. Cattle will be infected with FMDV SAT 1, 2 and 3 virus using intranasopharyngeal (INP) inoculation system. ELISPOT will be used for detection of FMDV-specific antibody-secreting B cells. Multiplex staining panel developed to differentiate cattle B and T cells into putative functionally distinct subsets will be used to characterize B and T cell response to FMDV infection. Furthermore, immune transcriptome regulation will be determined to characterize global immune response to FMDV using the RNA-sequencing technology. Understanding of transmission mechanism underlining persistent FMDV infection and activation of innate and adaptive immune response in livestock will assist in disease control strategies. Furthermore, immune transcriptome data will also assist in development of next-generation vaccines and antiviral products against FMDV.

Poster Session 3 – Monday 20 November

212 - Foot and Mouth Disease Vaccine Immunogenicity Study Among the Agro-Pastoralists In Plateau State, Nigeria

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Foot-and-mouth disease is endemic in Nigeria and has impacted negatively over the years on the national economy, nutrition, and means of livelihood of rural households in the country. Post-vaccination monitoring of a commercial trivalent (O, A, SAT 2) FMD vaccine was carried out to determine the field immunogenicity of the vaccine in cattle among the Ago-pastoralists in Bokkos, Local Government areas of Plateau State, Nigeria. All the cattle (n=100) in the herd were bled on day 0 before the first primer dose of the vaccine was administered to all the animals. On day 28 post-vaccination, all the cattle in the herd were subsequently bled and a booster dose of the vaccine was administered. On day 56 post-vaccination all the animals in the herd were again bled to check for evidence and duration of antibodies to FMD virus. The samples (n=45) were analyzed using serotype-specific (O, A SAT 2) Enzyme-Linked Immunosorbent Assay (ELISA) kits, according to the manufacturer's instructions. The result obtained from the analyzed samples showed that after primer dose post-vaccination, herd immunity levels of (96%,73%, and 100%) for serotypes O, A, and SAT 2 were developed respectively. After the booster dose at day 28 post-vaccination, herd immunity levels of (84%, 56%, and 100%) were observed respectively for serotypes O, A, and SAT 2. The study revealed good herd immunity for FMD in the vaccinated cattle population for serotypes O and SAT 2 except for serotype A. This information will improve our knowledge of the use of commercial FMD vaccines for the progressive control of the disease in Nigeria.

Poster Session 3 – Monday 20 November

Theme 6. Immunology of bacterial diseases

098 - IgM antibodies play a major role in the elimination of *Streptococcus suis* serotype 2

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The adaptive humoral response is the result of a communication network between antigen presenting cells (APC), T cells and B cells. Antibodies play a useful role in the elimination of *Streptococcus suis*, an encapsulated bacterium that can cause severe invasive disease in pigs. However, no commercial vaccine is available to prevent infections. Reports indicate that *S. suis* can interfere with optimal APC and T cell functions. However, the interactions between *S. suis* and B cells are largely unknown. The aim of this study was to characterize the development of the adaptive humoral immune response by evaluating GC B cell dynamics and the production and role of antibodies induced following *S. suis* infections in a mouse model. We found that mice infected with *S. suis* developed GC that peaked 13-21 days post-infection. GC further increased and persisted upon periodic reinfection that mimics real life conditions in swine farms. Anti-S. suis IgM and several IgG subclasses were produced, whereas antibodies against the *S. suis* capsular polysaccharide (CPS) were largely IgM. Somatic hypermutation or isotype switching were dispensable for controlling the infection or anti-CPS IgM production. Depletion of total IgG from the WT mice sera had no effect on bacterial killing in vitro. However, T cell-deficient (Tcrb-/-) mice were unable to control bacteremia, producing optimal anti-CPS IgM or eliciting antibodies with opsonophagocytic activity. SAP deficiency, which prevents GC formation but not extrafollicular B cell responses, ablated anti *S. suis*-IgG production but maintained IgM production and eliminated the infection. In

contrast, B cell deficient mice were unable to control bacteremia. Collectively, our results indicate that a GC-independent but T cell-dependent germline IgM being the major effective antibody specificity. Our results further highlight the importance IgM and potentially anti-CPS antibodies in clearing *S. suis* infections and provide insight for future development of *S. suis* vaccines.

Poster Session 1 – Saturday 18 November

099 - *Streptococcus suis* surface-antigen recognition by antibodies and bacterial elimination is influenced by capsular polysaccharide structure

Dominic Dolbec[1], Mélanie Lehoux[1], Masatoshi Okura[2], Daisuke Takamatsu[3], Marcelo Gottschalk[1], Mariela Segura[1]

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Streptococcus suis is an encapsulated bacterium that can cause severe invasive diseases in pigs. The bacterial capsular polysaccharide (CPS) is a critical virulence factor that provides resistance against host phagocytic cells. The antigenicity of the CPS defines 29 distinct serotypes of *S. suis*, with some serotypes being more commonly associated with clinical disease than others. For instance, the serotype 2 is the most prevalent worldwide. Our hypothesis was that the structure of the CPS influences survival in the host and resistance against antibodies targeting subcapsular antigens (such as proteins) at the bacterial surface. Therefore, serotype-switched mutants of *S. suis* serotype 2 were employed to compare the role played by the CPS structures of serotypes 2, 3, 4, 7, 8, 9 and 14, since the only difference between these strains is the CPS expressed. Primary and secondary infections in a mouse model showed that strains expressing the CPS of the serotypes 3 and 4 were the most susceptible to host defences during a primary infection. During the secondary infection, strains expressing the CPS of serotypes 3, 4 and 14 were the most eliminated. Furthermore, CPS structure was found to influence antigen recognition by antibodies. The CPS of serotypes 3, 4 and 14 allowed more IgG binding to subcapsular antigens (such as proteins) than the CPS of serotypes 2, 7, 8 and 9. This feature consequently affected antibody capacity to induce opsono killing of *S. suis*. Results suggest that the different CPS structures of *S. suis* provide varying levels of protection by influencing antigen availability and elimination by the host immune system. This finding is of importance for vaccine development and highlights the need to closely monitor cross-protection when designing *S. suis* vaccines since the CPS structure might eventually affect the efficacy of vaccines targeting subcapsular antigens at the bacterial surface.

Poster Session 1 – Saturday 18 November

Theme 7. Immunology of parasite diseases

028 - Ovine resistance against *Haemonchus contortus*: Does a breed or a β-globin subtype feature?

Cintia H Okino[1], Hornblenda JS Bello[1], Rafaela TI Kapritchkoff[2], Gláucia R Melito[1], Maria Isabel M Fiorentino[1], Flávia AB Donatoni[1], Simone CM Niciura[1], Alessandro P Minho[1], Sérgio N Esteves[1], Ana Carolina S Chagas[1]

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Background: Hair sheep breeds, as Santa Inês, are well-known for improved natural resistance and/or resilience against gastrointestinal infections, especially by Haemonchus contortus, compared to wool breeds, such as Dorper and Texel. Several studies have also pointed out to significant association of resistance against hemonchosis and β-globin polymorphisms.

Objectives: Compare phenotypic, Th2 profile and inflammatory responses during infection with H. contortus in different sheep breeds (Santa Inês-SI, Texel-TX and White Dorper-DO) harboring different β-globin haplotypes (AA, AB, BB).

Methods: Four male lambs of each group (SI-AA, SI-AB, SI-BB, TX-BB and DO-BB) were dewormed and, after 14 days, received 4000 L3 H. contortus (D0). Animals were weekly evaluated for packed cell volume (PCV), fecal egg counts (FEC) and blood gene expression until D28.

Results: For DO and TX, there were no AA animals in the flock, while rare AB were found, therefore they were not included in this study. Comparing BB haplotype lambs, all three sheep breeds presented similar PCV. However, SI-AA lambs presented significantly higher PCV compared to DO-BB and TX-BB from D7 to D21, and to SI-BB on D21. Despite no significant differences, higher FEC levels were observed for DO and lower levels for SI-AA. Differential Th2 profiles were observed among groups, higher IL4/IL13 levels were detected in SI animals (especially SI-AA), while IL5 was most prominent in TX and secondly in DO. Inflammatory response was IL1B polarized in SI, but TNFA in TX. Significant higher levels of MS4A2 (high affinity IgE receptor gene) were observed in SI breed.

Conclusion: Based on similar PCV values among SI-BB, DO and TX groups, but superior PCV for SI-AA animals, we hypothesized that resistance against hemonchosis may be more associated to the β-globin subtype than to the breed feature. Differential Th2 and inflammatory responses were also distinct among these groups.

Poster Session 1 – Saturday 18 November

191 - Evaluation of global immune responses in goats infected with *Haemonchus contortus* using RNA sequencing

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Haemonchus contortus is an extremely pathogenic resident gastrointestinal nematode in tropical and subtropical regions worldwide, causing significant production and economic losses in small ruminants. During infection, this nematode feed by sucking blood from the abomasum mucosa. Over the years, anthelmintic drugs have been used to manage this nematode; however, drug resistance is becoming a problem. There is only one registered vaccine (Wirevax®) in South Africa, and it is approved for use in sheep. Therefore, there is an urgent need to develop alternative vaccines for goats as well. *H. contortus* infection is usually associated with the Th2 immune responses. However, the detailed host immune mechanisms and pathways involved in protection against *H. contortus* infections are not well-understood. This study aims to evaluate the immune response mechanisms and pathways involved during *H. contortus* infection in goats using RNA sequencing (RNA-seq). To achieve this, goats will be orally infected twice with *H. contortus* L3 larvae in five-week intervals. The innate and adaptive immune responses following primary and secondary infection will be evaluated in peripheral blood mononuclear cells (PBMC) separated using magnetic-activated cell sorting (MACS). Immune transcriptome analysis will be done in individual cell populations using RNA-seq. This will provide insights into the immune mechanisms and pathways involved in goat immune responses to *H. contortus.* Understanding the immune response mechanisms and pathways involved will lay a foundation for developing alternative vaccines against *H. contortus.*

Poster Session 1 – Saturday 18 November

227 - A Comparative immunological profile of *Babesia microt*i infected BALB/c mice co-infested with *Ixodes ricinus* ticks and its effect on immunisation

Nicola Greyling[1], Luïse Robbertse[1,2], Christian Stutzer[1], Christine Maritz-Olivier[1]

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Parasites have evolved a variety of mechanisms to evade or manipulate their host's immune responses. Co-infestation (or infection) with multiple pathogens and/or parasites is common in animal hosts under field conditions. Such co-infestations have the potential to modulate the host immune responses to make it more susceptible to other infections/infestations, and likely confound the efficacy of therapeutic vaccines. A greater understanding of the immunological interplay elicited during co-infestation of a host animal model, with parasites of economic importance, could therefore influence the way in which next generation vaccines are designed. Consequently, a pilot study was conducted using a co-infection model with *Babesia microti* and *Ixodes ricinus* ticks in BALB/c mice that was interrogated in the presence and absence of immunisation with a formulated mock vaccine. Results show that co-infestation of *I. ricinus* and *B. microti* skews host immunity towards a Th1-meadiated immune response with a down-regulation in antigen specific IgE antibodies. Both B- and T lymphocyte data indicated that *I. ricinus* ticks have a negligible effect on the lymph node, spleen and blood lymphocyte subpopulations, likely due to the immunosuppressive effect of the tick saliva. Furthermore, mice infested with only *B. microti* or co-infested with ticks activated similar host lymphocyte subpopulations. Tick co-infestation also resulted in a significant up-regulation of anti-inflammatory cytokines, IL-10 and TNF-α, that can limit the host's response to both parasites. This study is a first attempt to describe the effects that a tick vector and its associated pathogen has on a model host and how these simultaneous burdens can influence antigen-specific host immune responses.

Poster Session 1 – Saturday 18 November

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228 - *In vivo* evaluation of *Ixodes ricinus* induced effects on T and B-cell maturation in the spleen and lymph nodes of BALB/c mice

Christian Stutzer[1], Luïse Robbertse[1,2], Christine Maritz-Olivier[1]

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Despite the inability of the BALB/c mice to acquire resistance against *Ixodes ricinus* nymphal attachment after multiple infestations, immune responses in naïve animals to tick infestations are inevitable. To investigate this, our study aimed to characterise the *in vivo* response of tick-naïve and nymph extract immunised BALB/c mice infested with *I. ricinus* nymphs. This was done by characterising B and T-lymphocyte populations in the draining lymph nodes and spleens of these BALB/c mice. Additionally, the T-helper 2/ T-helper 1 cytokine ratios from in vivo isolated lymphocytes from tick-infested mice were evaluated. Results indicate that immunising BALB/c mice with nymph extract does not provide significant protection to subsequent tick infestations. Additionally, a B-cell population (CD45+ CD19+ IgM+ CD27+CD80+) was uniquely up-regulated during tick feeding and significantly down-regulated in immunised mice at 9- and 8-days post infestation, respectively. Regarding T-cells, no significant differences in the numbers of CD45+ CD3+ CD4+ T-helper cells were noted in tick-naïve, or nymph extract immunised mice compared to their controls. Concurrently, a T-helper cell subset (CD45+ CD3+ CD4+ CD195+ CD184-) was significantly decreased in tick-naïve mice at 12 days post infestation. A slight significant decrease in T regulatory cells (CD45+ CD3+ CD4+ CD25+) was observed in only nymph extract

immunised mice. The results presented in this study are the first to describe populations of T and B-lymphocytes in the lymph nodes of tick extract immunised and tick-naïve mice in response to infestation. Although additional studies with a finer sampling time scale and *in vitro* stimulation of lymphocytes are needed to further describe the observed lymphocyte populations, the results presented indicate that unique cell populations are affected by tick extract immunisation and tick feeding.

Poster Session 1 – Saturday 18 November

Theme 8. Vaccine Development

014 - Comparison of immunogenicity of vaccine payloads delivered by 3 different viral vaccine vectors

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As initiatives to develop and test new vaccines for infectious diseases of livestock become more critical in the face of pathogens developing resistance to antibiotics, there are now many choices for vaccine delivery platforms. However, there is little published data available that directly compares the performance of different platforms. Such data would enable an informed development of new vaccine payloads and assays for analyzing immune responses to vaccination. We have a variety of vaccine platforms that have been tested for their efficacy in payload delivery and ability to elicit immune responses in ruminants. These include replication defective adenovirus 5 (Ad5), a vector derived from the small ruminant lentivirus maedi-visna virus (MV), and an attenuated Alcelaphine gammaherpesvirus 1 (AlHV-1). In our development of next generation vaccines for bovine respiratory disease (BRD) syndrome, we have initially focused on the viral pathogens of BRD. We have cloned the bovine herpesvirus 1 (BHV-1) glycoprotein B (gpB) and bovine respiratory syncytial virus (RSV) glycoprotein G (gpG) into these delivery platforms. These vectored vaccines were all shown to express the vaccine payload in vitro. Subsequently, cohorts of cattle were vaccinated with one of these vaccine vectors, specifically Ad5 delivering the BHV1-gpB (Ad5gpB) as a payload, and the resulting humoral response has been shown to be positive by ELISA. We now aim to investigate the remaining vectors and payloads to assess the performance of the different platforms in direct comparison studies. This will provide preliminary data to indicate whether any of these vector platforms are more efficient at stimulating antibody responses to these viral antigens in cattle. These results will inform the design of follow-on studies that compare the efficacy of vaccination with a single vector expressing multiple antigens versus different vectors delivering different antigens.

Poster Session 1 – Saturday 18 November

039 - The development of a subunit vaccine for the control of crocodilepox virus infection in farmed Australian saltwater crocodiles (*crocodylus porosus*)

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Saltwater crocodiles (*Crocodylus porosus*) are farmed mainly in Northern Australia to produce skins for the international leather industry, with Australia accounting for 60% of the global trade in saltwater crocodile skins. However, only skins without defects on the abdominal region are ideally suitable for export, with the remainder being downgraded, resulting in a loss of profit for the producer. Major viral, bacterial, fungal and parasitic pathogens affect the quality of the belly skin. In particular, crocodile poxviruses have been reported to infect a number of different crocodilians, including *C. porosus*. Crocodilepox infection results in lesions comprising the underlying dermal structures that impact the quality of leather. Currently, there are no specific treatments or vaccines available. We set out to develop a vaccine to protect farmed crocodiles from infection by crocodilepox. Potential antigens were selected based on homology to proteins in other pox virus antigens producing neutralising antibodies. Four antigens were selected cpB5, cpA17, cpH3L and cpL1, which were selected for expression and purification in *E. coli*. A trial aimed to assess the efficacy of the quil A-adjuvanted subunit vaccine in a substantial population of yearling crocodiles under field conditions was conducted on a farm known for recurrent crocodilepox outbreaks. Groups of 35 crocodiles received two intramuscular or subcutaneous vaccinations four weeks apart. Serum was collected from a subset of the vaccinated and unvaccinated crocodiles before and after vaccination to evaluate the humoral response to vaccination. All antigens induced a strong immune response in the crocodiles, indicating that the recombinant proteins were immunogenic and have the potential as vaccine candidates. However, the ability to assess the efficacy of the vaccine candidates during natural 'on-farm' infection was impeded due to crocodilepox outbreak occurring very early during the trial, potentially before immunisation of the animals, or very early after the first vaccine dose.

Poster Session 1 – Saturday 18 November

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040 - Induction of humoral and cellular responses to parapox virus-vectored antigen for vaccine development

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Virus vectors have wide applicability in vaccinology for their ability to stimulate both humoral and cellular immune responses in a range of species. Orf, a parapox virus that naturally infects sheep and humans, is a good virus vector candidate as immunity is not long-lived, thereby reducing concerns of anti-vector responses. *Chlamydia abortus* is an intracellular bacterial pathogen responsible for causing ovine enzootic abortion (OEA) in most countries worldwide. There is a need for a safer, more efficacious vaccine. Thelper-1-type cellular responses are known to be strongly associated with protection to *C. abortus*. Here, we describe the cellular and humoral immune responses elicited in sheep immunized with a modified Orf virus vector (OrfV) containing the major outer membrane protein (MOMP) of *C. abortus*, a known protective chlamydial antigen.

Aim: To assess the potential use of OrfV-MOMP as a vaccine to protect sheep from OEA.

Methods: Thirty OEA-free sheep were allocated into three groups of 10. These were immunized intramuscularly with: 1) live OrfV-MOMP; 2) inactivated OrfV-MOMP; and 3) an unvaccinated control group. Serological responses were evaluated by measuring anti-MOMP IgG and cellular responses were evaluated by

measuring recall responses of PBMC from immunized animals to whole killed chlamydial organisms and a subunit antigen preparation containing MOMP.

Results: Immunization with either live or inactivated OrfV-MOMP induced anti-MOMP IgG that was not detectable in the unvaccinated control group. Antigen-specific recall responses, characterised by the secretion of interferon-gamma and interleukin (IL)-17A, with very low levels of IL-10 and no IL-4 detected in both immunized groups, suggesting induction of an appropriate immune response to control infection.

Conclusions: OrfV-MOMP induces humoral and cellular immune responses that are desirable for vaccine-induced protection against *C. abortus* infection in sheep.

Poster Session 3 – Monday 20 November

047 - Immune response against bacterins used to control *Streptococcus suis* infections in pigs

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Restrictions in the use of antibiotics brought an increase of clinical disease in nursery piglets. *Streptococcus suis* is probably the most important bacterial pathogen affecting these pigs and is also a zoonotic agent of critical concern mainly in Asia. There is no commercial vaccine and the only alternative practitioners have is the use of autogenous vaccines (bacterins) based on the predominant strain(s) recovered from diseased pigs in the affected farm. The objectives of the current study were to evaluate the effect of following parameters on the immune response and protection induced by a *S. suis* bacterin: a) different adjuvants; b) the use of a concentrated bacterial supernatant added to the bacterin formulation; c) the use of a single strain vs the combination of several strains (belonging to different serotypes, a common practice in the field). From the six adjuvants tested, some of them did not induce any antibody response and were non-protective. Among them, adjuvants such as Alhydrogel and Emulsigen D, are currently used in the field by many autogenous vaccine companies. Oil-based emulsions presented low (oil-in-water) or high (water-in-oil) immune response and protection, depending on the adjuvant. Adding concentred supernatant to the bacterin did not increase protection, even in the presence of higher levels of the hemolysin (suilysin), previously described as being protective. Using a water-in-oil adjuvant, a multivalent (five different serotypes) was as effective as a monovalent bacterin, suggesting that the use of multiple strains in a bacterin (commonly used in the field) does not affect the protective capacity of the vaccine. In theory, producing a killed bacterin is not complicated. However, there are so many ways to produce such a vaccine that it is literally impossible to compare autogenous vaccines produced by different laboratories. A better standardization of bacterin production will benefit the swine industry.

Poster Session 3 – Monday 20 November

048 - Clinical assessment and transcriptome analysis of host immune responses in a vaccination-challenge study using a glycoprotein G deletion mutant vaccine strain of infectious laryngotracheitis virus

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Infectious laryngotracheitis (ILT) is a respiratory disease of poultry caused by infectious laryngotracheitis virus (ILTV). Infection results in morbidity, mortality and reduced egg production in affected flocks, and leads to significant economic loss to poultry industries worldwide. Currently, the disease is controlled by live-attenuated vaccines. A glycoprotein G (gG) deletion mutant live-attenuated vaccine strain of ILTV (ΔgG ILT) has been shown to be safe and efficacious in previous *in vivo* studies. However, the immune signatures of ΔgG ILT vaccination, or the specific genes and/or pathways involved in vaccine efficacy remain unclear. In the current study, an assessment of clinical outcomes and an analysis of the transcriptomic response of specific pathogen-free chickens were compared between unvaccinated or ΔgG ILT vaccinated birds after challenge with virulent ILTV. Significant differences in mortality rate, clinical scores, tracheal pathology scores (gross and microscopic), percentage weight gain and ILTV viral load detected via qPCR, clearly demonstrated that the administration of the ΔgG ILT vaccine to chickens resulted in clinical protection against challenge. In addition to this, a combined assessment of the transcriptome of the blood-derived monocytes at 7 days post-vaccination and of tracheal scrapings at 4 or 5-days post-challenge, was undertaken to describe the transcriptome-level differences between the vaccinated-challenged and the challenged-only group.

Poster Session 3 – Monday 20 November

053 - New advances in the development of a subunit vaccine targeting antibody production against African swine fever

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African swine fever (ASF) is a highly contagious viral disease affecting domestic pigs and feral hogs with mortality rates near 100%. Although the disease entered Georgia in 2007 from Africa, where the disease in endemic, the attempts to control the disease where unsuccessful and it spread through the European and Asian continent, and most recently to the Caribbean. Part of the challenge to control ASF is the lack of vaccine, counting only with biosecurity, rapid diagnostic and culling of infected animals as control measures. The causative agent of ASF is a double stranded DNA virus (ASFV) whose genome encodes more than 150 different proteins. Different attempts to develop a vaccine had variable efficacy outcomes, with the most promising approaches being the generation of attenuated recombinant viruses. However, they pose several limitations, such as biosecurity, need of cold chain and difficult production. The development of subunit vaccines against ASF will overcome these limitations. However, from the 150 ORF the ASFV genome codes, only a few have been identified as capable to induce protective antibodies and so far, the protection afforded has been rather limited. DNA libraries are very helpful to identify new antigens because of their capacity to represent all the pathogen antigens. They are also valuable tools to apply in in vivo immunogenicity studies. We designed a DNA library encoding all the ASFV-Kenya1033 (Genotype IX) ORFs to screen the differential pattern of antigen recognition of serum antibodies from domestic pigs, susceptible to ASF, and warthogs, natural reservoirs of ASFV that are resistant to the disease. Together with a newly developed seroneutralization assay, we assessed the capacity of the antigen-specific antibodies to inhibit ASFV infection in vitro. The results from both assays led to the identification of a panel of new ASFV antigens that we tested under in vivo immunogenicity studies.

055 - Spontaneous Nanoliposome Antigen Particleization (SNAP) - a Novel Subunit Vaccine Technology for Livestock Vaccine Development

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The development of effective sub-unit vaccines offers promising opportunities for a sustainable and scalable solution to East Coast fever, a major disease affecting cattle in Eastern, Central, and Southern Africa. Recent experimental trials have shown that a recombinant sporozoite candidate vaccine antigen, p67C, provides 50% protection against the LD70 sporozoite needle challenge. In this study, we aimed to assess the efficacy of Spontaneous Nanoliposome Antigen Particleization (SNAP) in stimulating a potent immune response using p67C as a model antigen. Four groups of cattle were immunized with recombinant his-tagged soluble p67C mixed with different SNAP ingredients: cobalt-porphyrin-phospholipid (CoPoP), Phosphorylated Hexaacyl disaccharideCoPoP(PHAD); a TLR4 agonist, and QS21; a saponin, which were used individually or in combination of two or three ingredients. To evaluate the potency of the immunogen, mice were immunized with p67C mixed with CoPoP/PHAD/QS21 as well as p67C adjuvanted with Alum. The results showed that p67C antigen mixed with CoPoP/PHAD/QS21 and CoPoP/QS21 induced an IgG antibody response in cattle, while p67C mixed with CoPoP only and CoPoP/PHAD did not effectively elicit an IgG antibody response. In mice, p67C mixed with CoPoP/PHAD/QS21 generated a stronger IgG antibody response compared to Alum. Interestingly, the SNAP technology, in its current formulation, did not significantly increase p67C IgG titres in cattle, but it did demonstrate a two-fold increase in mice. This discrepancy could be attributed to inherent physiological and pathological differences between cattle and mice, considering that East Coast fever primarily affects cattle. Overall, this study highlights the potential of SNAP technology for vaccine delivery of poorly immunogenic antigens like p67C, with the need for further formulation improvements to optimize its application in large animals.

Poster Session 3 – Monday 20 November

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059 - Development of a novel recombinant subunit Q fever vaccine for ruminants

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Coxiella burnetti is the causative agent of Q-fever, a highly contagious zoonotic disease, which causes abortion, stillbirth and weak offspring in goats, sheep and cattle. Currently, the only existing commercial vaccine for livestock is COXEVAC® (CEVA Animal Health Ltd.), an inactivated whole bacteria based on the phase I virulent

form of C. burnetti. However, increased body temperature, decrease of milk yields and painful injection site reactions caused by the vaccine have been reported. Furthermore, the manufacturing of this vaccine requires the culture of the bacteria at Biosafety Level 3, making it not only highly costly, but also presenting significant health and safety issues. Hence, the development of a safer, effective, and easy to manufacture vaccine is essential. Using reverse vaccinology, we are working towards the identification of novel antigens for incorporation into a recombinant subunit vaccine. Initially, we used a high-density peptide microarray to identify potential antigens targeted by COXEVAC® through linear B-cell epitope mapping of the entire C. burnetii proteome with antisera from vaccinated sheep. From this work, we have identified six immunogenic proteins which have shown protection in an outbred mouse Q fever challenge model. In parallel to this, we aim to perform single cell B-cell receptor sequencing from Ag-specific B cells in sheep peripheral blood following immunisation with COXEVAC® to generate a panel of recombinant sheep monoclonal antibodies. These antibodies will be tested for functional activity in vitro, including neutralisation of cellular uptake and reduced intracellular replication. Antigen targets of functional antibodies will be elucidated through immunoscreening approaches and tested for protection in mouse models. Ultimately, the most protective vaccine formulations will be taken forward for efficacy testing in target ruminant species.

Poster Session 3 – Monday 20 November

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060 - Virally-vectored immuno-contraceptives for the management of feral cats in Australia

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Since their introduction, the impact of feral cats on Australian native wildlife has been devastating. Feral cat numbers are estimated to be up to 6.3 million and they are responsible for killing approximately 75 million native animals a day. In Australia, their activity has been a driving force behind the decline, and even extinction, of many land-dwelling birds, reptiles and mammals. Virally vectored immunocontraception (VVIC) has been identified as a potential population control method for invasive vertebrate species including cats. The technique relies on stimulation of the host immune system to suppress either the occurrence or continuation of a pregnancy. Properly designed, immunocontraceptives have the potential to be more humane and effective, while requiring less human input with regards to delivery than current population suppression methods. I will present work detailing the construction of feline immunocontraceptive candidates derived from feline herpesvirus (FHV-1) which contain critical reproductive genes essential for reproduction-related processes in both male and female cats. We predict that viral expression of these genes will induce an immune-directed disruption of the natural activity of both of these genes, rendering the cat partially or fully sterile. This project is expected to provide new insights into the use of VVIC control in Australia for large-scale population control of invasive vertebrate species.

Poster Session 3 – Monday 20 November

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069 - Investigating the soil mycobacteriome to validate M. bovis tuberculin skin test results

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The environment (soil and water) contains a diverse mycobacteriome which can influence mycobacterial infection of hosts and their immune responses. The soil mycobacteriome is composed primarily of non-tuberculous mycobacteria (NTMs) which are saprophytes or opportunistic pathogens. Pathogenic Mycobacteria tuberculosis complex (MTBC) including M. bovis may also be present in the environment if shed by infected hosts. NTMs can elicit a host immune response which may result in cross-reactions to shared antigens of MTBC. Since immunological tests are widely used for the detection of M. bovis infected animals in South Africa, false positive results in high value animals, such as African buffalo (Syncerus caffer), can result in unnecessary economic and genetic losses. Therefore, it is important to investigate suspect TB diagnostic test results and identify NTMs in the environment that may lead to immune sensitization. In this pilot study, the diversity of Mycobacteria spp. within the soil microbiome was evaluated using molecular techniques in an area where suspect tuberculin skin test reactor buffaloes have been reported (M. bovis historically free area). The DNA was extracted from mycobacterial cultures of environmental samples, followed by PCR (rpoB and hsp65) amplification and Sanger sequencing to provide species level identification. In 31.8% (7/22) of the samples, M. bovis was present along with several NTM species. This indicated that although some of the NTMs detected may result in cross-reactivity, the presence of environmental M. bovis was a more likely cause of immune sensitization, reflected by a positive tuberculin skin test result. The molecular techniques used in this study could successfully investigate the soil mycobacteriome and would be recommended for investigating future instances of suspect tuberculin skin test reactor buffaloes.

Poster Session 3 – Monday 20 November

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070 - Evidence of glycosylation in the schizont life cycle stage of ***Theileria parva*** parasites

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The apicomplexan parasite *Theileria parva* (*Tp*) causes East Coast Fever, the most important tick-borne disease of cattle in sub-Saharan Africa. Disease prevention relies on the Infection and Treatment Method (ITM), which has several limitations including parasite strain-restriction of induced immune reactions in treated cattle. Constant improvement of carbohydrate-based vaccines and the importance of glycosylated molecules for closely related Apicomplexans such as *Plasmodium spp.* inspired this project to investigate the role of glycosylation in different life cycle stages of *Tp* in cattle. The first step was to confirm the presence of glycosylated parasite-derived molecules. Flow cytometric analyses of isolated *Tp* schizonts showed binding of recombinant bovine Macrophage C-type Lectin (MCL) to the parasites, potentially targeting glycan ligands on the schizont surface. Strong binding of the plant lectin Wheat Germ Agglutinin (WGA) to the parasites, inhibitable in a dose-dependent manner, further indicated the presence of N-Acetylglucosamine (GlcNAc) residues on surface molecules of *Tp* schizonts. Western Blot analyses of the whole protein extract (WPE) from *Tp* schizonts highlighted a clear reduction in WGA staining after peptide-N-glycosidase (PNGase) F treatment of WPE and suggested that the GlcNAcylated molecules could be parasite-derived N-glycoproteins. A glycopeptide analysis to confirm parasite origin of and further characterise GlcNAcylated proteins is currently ongoing. To the best of our knowledge, these results represent first evidence of glycosylation in *Tp* and support the detection of genes encoding three glycosyltransferases in the parasite genome by Tretina *et al.* in 2020. Expanded knowledge on the glycosylated molecules, structures of modified glycans and their roles in host cell transformation as well as parasite survival in bovine lymphocytes will help us to identify novel vaccine targets and ultimately design a carbohydrate-based vaccine to fight East Coast Fever.

074 - Transcriptomic profiling shows the induction of humoral and cellular response-related genes in pigs following vaccination with an Influenza A nanovaccine

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Influenza A virus (IAV) causes a major economic concern for the swine industry and represents a pandemic threat for humans. Vaccination with IAV strains is the main strategy to control the disease in pig herds. Although some influenza vaccines were developed and tested in field trials, molecular mechanisms underlying nanovaccine-induced protection are still unknown. Here, we evaluated the gene expression profile in mediastinal lymph nodes (LMD) of 8 non-vaccinated specific-pathogen free pigs and 16 vaccinated pigs with an adjuvanted nanovaccine for IAV containing the surface glycoproteins (hemagglutinin and neuraminidase) of H1N1pdm, H1N2 and H3N2 viruses. The LMD of the 24 pigs were submitted to the RNA-Seq analysis with the Truseq Stranded mRNA (Illumina) and sequencing in NextSeq 2000 sequencer (lllumina) using 2x100bp paired-end reads protocol. Reads were submitted to quality control using Trimmomatic, mapped and counted with STAR against the swine reference genome (Ensembl 109) and analyzed with limma package considering differentially expressed (DE) genes when a false discovery rate (FDR) was <0.05 and a logFC > |1.5|. The functional annotation of the DE genes was performed using DAVID database. Seventeen genes were DE, all of them upregulated in the vaccinated group, and enriched biological processes (BP) involved with positive regulation of cell cycle. Among them, several genes were related to humoral (GCSAM, MYBL1, MYBL2, ELL3, SIPR2) and cellular (AFF2, POU2AF1, ASF1B) immune response. Furthermore, BP involved with cellular to lipid (AICDA, NUGGC, SCIMP) were also identified, relating them to the phospholipid envelope of the influenza virus present in the nanovaccine. Therefore, in this study, the gene expression profile involved with a virosome-based vaccine immunogenicity was highlighted, showing significant activation of humoral and cellular responses through different biological processes that are related with virus clearance and sustained protection.

Poster Session 3 – Monday 20 November

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078 - Identification of new antigens as potential sub-unit vaccine candidates for ECF control

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East Coast fever (ECF), caused by the *Theileria parva* parasite, is a fatal lymphoproliferative disease that affects cattle in East, Central, and Southern Africa and leads to significant economic losses exceeding $300 million per year. Currently, the disease is controlled using a live vaccine known as the Infection and Treatment Method (ITM) where animals are infected with the parasite and simultaneously treated with long acting oxytetracycline, an antibiotic. Although effective, the vaccine has various limitations, it is expensive, takes a long time to manufacture, can lead to antimicrobial resistance, can result in a lifelong carrier state, and requires a cold chain for delivery. To overcome these limitations, there have been efforts to develop a sub-unit vaccine mostly focusing on p67C; the major surface antigen of the sporozoite stage of the parasite. Using p67C as a unique antigen in different formats the level of protection could not improve beyond 50% against an LD70 challenge. In an attempt- to enhance the protective efficacy of the subunit vaccine, additional sporozoite antigens will be incorporated into the vaccine formulation. The selection of the best cocktail of antigens is being done using a new *T. parva* sporozoite seroneutralization assay using archived sera against p67N and four newly discovered sporozoite antigens, namely TpSp1, TpSp2, TpSp3 and TpSp4. Here we present the selection of the best antigens to be included in a subunit vaccine using a recently optimized sporozoite seroneutralization assay.

Poster Session 3 – Monday 20 November

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097 - Influence of maternal antibodies on the immune response of young piglets vaccinated with a ***Streptococcus suis*** serotype 2 bacterin

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Streptococcus suis is the most important bacterial pathogen affecting post-weaned piglets and is also a zoonotic agent. There is no commercial vaccine and the only alternative practitioners have is the use of autogenous vaccines (bacterins) based on the predominant strain(s) recovered in the affected farm. Depending on the farm, sows or young piglets are vaccinated; in the latter case, it is a common practice to vaccinate them at 1 and 3 weeks of age (WOA) to protect them during the nursery period (from 4 to 10 WOA). Although an interference effect with maternal antibodies (MA) was suggested, it has not been proven yet. The objectives of the present study were: a) to compare the immune response of piglets vaccinated at 1 and 3 WOA (in the presence of high MA levels) vs those vaccinated at 3 and 5 WOA (lower MA levels); b) to use a newly developed model of colostrum-deprived conventional piglets (CDCP) to evaluate the interference of MA with a bacterin-based vaccination of piglets at 1 and 3 WOA. Results showed a clear antibody response in piglets vaccinated at 3 and 5 weeks of age. On the other hand, piglets vaccinated at 1 and 3 weeks of age induced a stabilization of the decay of MA when compared to control animals. Indeed, the sum of MA and active response had as a consequence that levels of antibodies in piglets at 7 and 9 weeks of age were not statistically different between the two groups of vaccinated piglets. By using the CDCP model, it was demonstrated that the presence of MA partially interferes with the active production of antibodies; however, as in the first study, the stabilization of the decay of MA seems to be as effective as an active immunization at 3 and 5 weeks of age.

Poster Session 3 – Monday 20 November

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100 - Immunogenicity and Protective Efficacy of Ag85A and truncation of PstS1 fusion protein vaccines against tuberculosis

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Tuberculosis is an important public health problem and the One Health approach is essential for zoonotic tuberculosis control. For that purpose, a rationally designed and more effective TB vaccine is urgently needed. For enhancing vaccine efficacy, it is important to design vaccine candidates that stimulate both cellular and humoral immunity against TB. In this study, we fused the secreted protein Ag85A as the T cell antigen with truncated forms of the mycobacterial cell wall protein PstS1 as B cell antigens to generate vaccine candidates named Ag85A-tnPstS1 (AP1, AP2, and AP3), and tested their immunogenicity and protective efficacy in mice. The vaccine candidates could induce significant increase in the levels of T cell-related cytokines such as IFN-γ and IL-17. Strong humoral immune responses were also observed in which the production of IgG antibodies including its subtypes IgG1, IgG2c, and IgG3 was tremendously stimulated. Importantly, the mice immunized with the subunit vaccine candidates, particularly AP1 and AP2, had lower bacterial burdens than the control mice. Moreover, the serum from immunized mice can enhance phagocytosis and phagosome-lysosome fusion in macrophages, which can help to eradicate intracellular bacteria. These results indicate that the subunit vaccines Ag85A-tnPstS1 can be promising vaccine candidates for tuberculosis prevention.

Poster Session 3 – Monday 20 November

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102 - Safety and efficacy of the Rift valley fever live-attenuated vaccine candidate 40Fp8 in pregnant ewes

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Rift Valley fever (RVF) is an arboviral disease that causes abortions and deaths in livestock (cattle, sheep, camels and goats) and wild ruminants, also affecting humans. Live-attenuated vaccines (LAVs) are ideal candidates for livestock vaccination programs, since they induce good levels of immunity with one single shot. However, the use of LAVs in immunocompromised hosts or pregnant animals is limited due to the possibility of reversion and/or the presence of residual virulence. We have obtained an RVFV mutagenized variant (40Fp8) that exhibited an extremely attenuated phenotype in immunodeficient murine models (IFNAR KO mice), nevertheless retaining its immunogenicity. In adult sheep 40Fp8 induced immunity levels which correlated with protection in the absence of clinical disease, thus making it suitable for vaccination purposes. The aim of this study was to test both safety and efficacy of 40Fp8 in a highly sensitive host such as pregnant sheep in their first third of pregnancy. After subcutaneous inoculation with an overdose (10^7 pfu) of this vaccine candidate, the ewes showed no clinical signs or reproductive failures leading to foetal death or miscarriage. A clear neutralizing antibody response at levels correlating with protection was detected from day 7 pi. Viral load in samples collected indicated very low levels of replication. Necropsies performed on days 4, 7, 14 and 30 pi showed no relevant macroscopic lesions in main target organs, and the developmental parameters of the foetuses were as expected for their gestation period. To test efficacy, sheep immunized following the same schedule were challenged with a high dose of a virulent RVFV strain and euthanized at 4-,

7- and 21-days post-challenge. Preliminary data showed no adverse effects in vaccinated ewes, in contrast to mock-vaccinated animals that showed liver injury, endometritis, foetal death, resorption and miscarriage, confirming the protective effect of our vaccine candidate.

Poster Session 3 – Monday 20 November

103 - Characterization of the efficacy of a split swine influenza A virus nasal vaccine formulated with a nanoparticle/STING agonist combination adjuvant in conventional pigs

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Novel and effective swine influenza A virus (SwIAV) vaccines are essential to reduce the disease burden in swine herds and risk for public health. Intranasal vaccination has the potential to reduce infection and transmission to susceptible animals, but there are few effective adjuvants for intranasal vaccines. Our objective is to investigate the efficacy of split SwIAV H1N2 antigens adsorbed with a plant-origin nanoparticle adjuvant (Nano-11) (Nano11-SwIAV) or in combination with the synthetic stimulator of interferon genes (STING) agonist ADU-S100 (NanoS100-SwIAV) in pigs. Conventional pigs were vaccinated twice intranasally and challenged with a virulent heterologous SwIAV H1N1-OH7 or 2009 H1N1 pandemic virus and euthanized a week later. An increased number of CD172a+CXCL10+CD80/86+ myeloid cells was detected in the blood of both Nano11-SwIAV and NanoS100-SwIAV vaccinates after challenge with SwIAV H1N1-OH7. The frequencies of CD49d+IL-17A+ T-helper/memory cells and cytotoxic T-lymphocytes were increased in the tracheobronchial lymph nodes of pandemic virus challenged animals inoculated with the Nano-11 particle-based vaccines, while the frequencies of CD49d+IFNγ+ T-helper/memory and cytotoxic T-lymphocytes were increased in the blood of pandemic virus challenged NanoS100-SwIAV vaccinates. Animals vaccinated with both Nano-11-based vaccines had significantly increased cross-reactive secretory IgA in mucosal secretions and serum IgG against SwIAV H1N1 and H3N2. This was associated with substantial reduction in the challenge virus load in nasal secretions. Thus, despite vast genetic differences between the vaccine H1N2 and both H1N1 challenge viruses, intranasal vaccination with NanoS100-SwIAV induced cross-protective immune responses in mucosal and systemic compartments. These findings strengthen the utility of this combination adjuvant in eliciting cross-protective antigen-specific cellular and mucosal immunity in swine.

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Poster Session 3 – Monday 20 November

104 - Isolation of porcine reproductive and respiratory syndrome virus glycoprotein specific monoclonal antibodies from hyperimmune pigs

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Porcine reproductive and respiratory syndrome viruses (PRRSV) continue to cause major economic losses to the global swine industry. The high degree of variability among circulating PRRSV isolates, facilitated by an inherent propensity for rapid mutation and evolution, poses a significant challenge to the development of effective vaccines. Antibodies capable of broadly neutralising PRRSV strains are of great interest as tools to guide next-generation vaccine design. The conserved neutralising epitopes on PRRSV glycoproteins, however, remain unknown, and our understanding of the mechanisms behind antibody-mediated neutralisation of PRRSV is incomplete. To help address this, we aimed to isolate PRRSV-neutralising monoclonal antibodies from pigs hyperimmunised by sequential exposure to heterologous PRRSV strains. Recombinant soluble forms of the PRRSV-1 minor envelope complex glycoproteins, GP2, GP3, and GP4, were produced using a mammalian cell expression system. Of the three, recombinant GP3 was most frequently recognised by antibodies in the serum from these animals. Fluorescently tagged GP3-tetramers were therefore constructed and used to isolate single B cells from the immune pigs by flow cytometry. IGL/IGG variable regions were amplified using a semi-nested RT-PCR workflow, sequenced, and complementarity-determining regions annotated using IgMAT. 15 unique heavy and light chain pairs were expressed as recombinant mAbs for evaluation of their PRRSV neutralising potency. Preliminary results have demonstrated the recognition of recombinant and native GP3 by these mAbs by ELISA and immunostaining of PRRSV-infected cells.

Poster Session 3 – Monday 20 November

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105 - Foot-and-Mouth disease SAT specific virus peptide phage display libraries for the identification of epitopes

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The WOAH ranks foot-and-mouth disease (FMD) as an economically important infectious animal disease affecting cloven hoofed animals. There are seven serologically distinct serotypes i.e. A, O, C, Asia1 and the Southern African Territories (SAT) types: SAT1, SAT2 and SAT3. Three of the seven serotypes exist in South Africa and considering the virus maintenance host i.e., the African buffalo, eradication is near impossible. Thus, emphasis is placed on control e.g., improved vaccines. Vaccination against one serotype does not confer protection against another due to high antigenic variation of the virus. Commonly, variations occur on the capsid coding (P1) region of the genome. Knowledge of the FMD virus (FMDV) antigenic sites can be useful in production of recombinant FMD vaccines. Due to limited knowledge regarding SAT antigenic sites, phage display technology was utilised. Consequently, three FMDV peptide phage display libraries were constructed using the fragmented P1 regions of a FMDV SAT1, SAT2 and SAT3 and biopanning with immunoglobulin (IgG) purified from FMDV SAT bovine infected sera. The advantage of utilising immune sera and biopanning against virus-specific peptide libraries is that affinity maturation has already occurred in immunized animals and the recognized epitope regions are identifiable. Antigenic regions to which IgGs bound were identified from screening biopanning output clones followed by Sanger and Miseq sequencing. The 18 amino acid residues from the VP1 C-terminus including three residues of the N-terminus of 2A was identified as a SAT3 epitope. The data also revealed other potential epitopic regions for SAT3. This study improved FMD knowledge on SAT antigenic sites and significantly contributes towards the future development of improved vaccines. Through recombinant, reverse genetics technology, identified epitopes can be incorporated into the FMDV genome

and recombinant viruses can be used for vaccine production, thus producing vaccines that offer a broad immunogenic response and protection.

Poster Session 3 – Monday 20 November

109 - Intranasal Ad5 influenza vaccine elicits hemagglutinin-specific antibody response in pregnant and lactating pigs

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Influenza A virus can cause severe complications for pregnant women and infants. New vaccines and strategies are being implemented to increase global access to vaccination in these vulnerable populations. Additionally, there are no influenza vaccines approved for infants younger than six months. While inactivated intramuscular (IM) vaccines are currently available for pregnant women, IM immunization may not be an ideal route to boost neutralizing specific antibodies in breastmilk. The aim of the study was to evaluate the capacity of a hemagglutinin (HA) (A/California/2009(H1N1)) Ad5 vector vaccine to induce specific passive immunity in pregnant and lactating pigs using different routes of administration. Pigs were used as a translational model to investigate the protective level of passive maternal antibodies in infants, after mucosal immunization. Influenza naïve pregnant pigs were vaccinated via oral or intranasal routes three weeks prepartum and boosted four weeks later (one week postpartum). Serum, colostrum and milk samples, as well as samples from the nasal mucosa and saliva were collected to measure the level of HA-specific antibodies induced by the vaccine over time in different mucosal tissues. Data showed that both HA-specific IgG and IgA antibody responses were induced by vaccination through the intranasal route in serum, colostrum and milk, but not after oral vaccination. Future research will evaluate the neutralization capacity of these HA-specific antibodies, as well as showing if this vaccine induces passive protection in a piglet challenge trial.

Poster Session 3 – Monday 20 November

117 - The pig as a biomedical animal model to develop treatment and prevention strategies against ***Chlamydia trachomatis***

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Chlamydia trachomatis (*Ct*) infections are the most common curable sexually transmitted infection leading to infertility, ectopic pregnancies, and chronic pelvic pain. In addition, *Ct* is the most prevalent infectious cause of blindness – trachoma. Both diseases are especially prevalent in sub–Saharan Africa. Besides humans, chlamydia poses similar health challenges in animals such as *C. suis* (*Cs*) in pigs. Based on the similarities between humans and pigs as well as their chlamydia species, we use pigs as a large biomedical animal model for chlamydia research. While the study of ocular infections is still in development, we successfully used the genital infection model for three purposes. First, we infected pigs genitally with *Cs* and *Ct* to study the induced immune response: as humans, pigs respond with a strong T-helper 1 response which is cross-reactive between *Cs* and *Ct*. Second, we performed a *Cs* vaccination and challenge study: this study could demonstrate both the immunogenicity and efficacy of the vaccine candidate showing that pigs can be used for chlamydia vaccine development. And third, we completed the first of three stages towards the development of a vaccine candidate against *Ct*: We not only showed that an IM/IN prime/boost vaccination is optimal but also that the "TriAdj" adjuvant optimally boosted the response to the chosen *Ct* protein vaccine antigen. Future studies will establish the trachoma model and further develop this *Ct* vaccine candidate by testing its immunogenicity and efficacy. Overall, this project shall open the bottleneck of large animal models to facilitate the progression of *Ct* vaccine candidates into clinical trials. In future, these vaccine candidates have promise for limiting the burden of ocular and genital infections in both pigs and humans. Based on this One Health approach, this project has the potential to profoundly impact the health of both pigs and humans.

Poster Session 3 – Monday 20 November

118 - Human whole-cell / B-subunit oral cholera vaccines Duochol and Dukoral induce antibody responses in a porcine model

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Oral cholera vaccine (OCV) is a critical tool in the WHO's global program for cholera control and elimination. Before testing new vaccines in humans preclinical testing in animals is performed. Pigs have some advantages over mice in that their anatomical size and structure, their immunology, genome, and physiology, are closer to these of humans, making them a more appropriate model to test an oral vaccination strategy intended to be used in humans than mice are. Pigs can for instance swallow capsule formulations unable to be swallowed by mice. Here a new low-cost, thermostable dry formulation of whole-cell / recombinant B-subunit (CTB) in enterocoated capsules (Duochol® OCV) was compared with the liquid commercial Dukoral® OCV. Both were administered three times with a 3-week interval to groups of 7 pigs. Serum IgG and IgA anti-Inaba and anti-Ogawa LPS as well as anti-CTB antibody responses were determined by ELISA and vibriocidal serum antibody levels against Vibrio cholerae O1 strains Inaba and Ogawa using guinea pig complement. Peripheral blood mononuclear cells were isolated to determine the antibodies in lymphocyte supernatant (ALS) response. Both Duochol and the liquid Dukoral were immunogenic in pigs, resulting in highly significant vibriocidal antibody responses to both Inaba and Ogawa bacteria in 7 out of 7 pigs that had received Duochol and in 6 of the 7 pigs who obtained Dukoral. There was also an immune response to CTB in most of the immunized animals however less impressive than the vibriocidal antibody responses, most likely due to preexisting antitoxin titers in some animals, probably a reflection to natural exposure to enterotoxigenic Escherichia coli. Duochol OCV was safe and showed equivalent immunogenicity as Dukoral. This is to our knowledge the first study ever in pigs to determine the immune response in pigs to any OCV whether liquid or capsule.

Poster Session 3 – Monday 20 November

121 - Protection of neonatal, colostrum-fed calves with a modified live, intranasal, tri-valent vaccine using an experimental challenge with virulent bovine respiratory syncytial virus

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Bovine respiratory syncytial virus (BRSV) is major viral contributor to bovine respiratory disease (BRD). Passive antibodies not only help protect the calf against infection but may interfere with the immune responses following vaccination. Pooled colostrum was administered by intubation to fifty-two (52) crossbred calves the day they were born. The calves were randomly assigned to be sham-vaccinated intranasally with a placebo or vaccinated with a tri-valent (bovine herpesvirus 1, bovine parainfluenza 3 and BRSV) modified live viral (MLV) vaccine. The calves were 7 to 11 days old when vaccinated (Day 0) at blood and nasal secretions collected over the next 80 days. The calves were challenged by aerosolized BRSV on Days 80 & 81 (~90 days of age) and clinical signs monitored, and blood and nasal samples were collected. The study was terminated on Day 88, animals necropsied, and the lungs evaluated and sampled. Rectal temperatures were significantly higher on days 5-8 post challenge in the control group. Cumulative respiratory scores were higher in the control group and one control animal died from BRSV. BRSV nasal virus secretion peaked at day 5 but the vaccinates shed 20-fold less virus. Nasal virus shed at day 8 post challenge was undetectable in the vaccinates and 60-fold less than the controls. Lung lesion scores (LLS) were significantly lower for vaccinated calves than those for control group. Both groups had similar serum neutralization (SN) antibody decay until challenge. Following challenge with BRSV, the vaccinated calves demonstrated a significant anamnestic response ($p<0.01$) on Day 88. After challenge, the calves sham-vaccinated lost weight while those vaccinated with the tri-valent MLV vaccine gained weight. In this study, colostrum-derived antibodies did not interfere with the immune response or protection provided by one dose of the IN MLV vaccine and the IN vaccine resulted in systemic humoral memory.

Poster Session 3 – Monday 20 November

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122 - Assessment of immunogenicity and efficacy of an attenuated herpesvirus-based vector expressing highly conserved porcine reproductive and respiratory syndrome virus M and NSP5 proteins

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Porcine reproductive and respiratory syndrome virus (PRRSV) remains a leading cause of economic loss to pig farming worldwide. Existing commercial vaccines fail to provide effective immunity against highly diverse circulating PRRSV strains. There is therefore an urgent need to develop more broadly protective vaccines. In

the absence of neutralizing antibodies, T-cells may play a central role in controlling PRRSV infection. Herpesvirus-based vectors are a novel vaccine platform capable of inducing high levels of T-cells against encoded heterologous antigens. The aim of this study was to assess the immunogenicity and efficacy of an attenuated bovine herpesvirus-4 (BoHV-4) vector expressing two well-characterized PRRSV-1 T-cell antigens (M and NSP5). Prime-boost immunization of pigs with BoHV-4 expressing the M and NSP5 (BoHV-4-M-NSP5) induced strong IFN-γ responses as assessed by ELISpot assays of peripheral blood mononuclear cells (PBMC) stimulated with a pool of peptides representing PRRSV-1 M and NSP5. A lower frequency of M and NSP5 specific IFN-γ responding cells was induced following a single dose of the BoHV-4-M-NSP5. Restimulation of PBMC with M and NSP5 peptides from PRRSV-2 demonstrated a high level of cross-reactivity. Vaccination with BoHV-4-M-NSP5 did not impact on viral loads in either the blood or lungs following challenge with two heterologous PRRSV-1 strains. However, BoHV-4-M-NSP5 prime-boost vaccination showed a marked trend toward reduced lung pathology following PRRSV-1 challenge. The limited effect of T-cells on PRRSV-1 viral load was further examined by analysis of local and circulating T-cell responses, both after vaccination and following challenge using intracellular cytokine staining and T-cell proliferation assays. Data suggest that the vaccine-primed T-cell responses may help in controlling PRRSV-1 associated tissue damage but had a minimal effect on controlling PRRSV-1 viral loads. Together, these results indicate that future efforts to develop effective PRRSV vaccines should focus on achieving a balanced T-cell and antibody response.

Poster Session 3 – Monday 20 November

127 - Construction of a FMDV-specific recombinant antibody phage-display bovine library for epitope identification and diagnostic reagents

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Foot-and-mouth disease (FMD) is caused by the foot-and-mouth disease virus (FMDV). It is the most wide-spread transboundary disease due to its highly infectious nature and is endemic in many African countries including, South Africa. There are seven serotypes of FMDV i.e., SAT1, SAT2, SAT3, A, O, C, and Asia1. The disease affects cloven-hoofed animals such as cattle, sheep, pigs and goats. Furthermore, it causes significant economic losses due to high morbidity in infected animals and stringent trade restrictions imposed on animal products from affected countries. An important control measure is vaccination, however, the FMDV high mutation rate results in antigenic variation rendering vaccines less effective. Knowledge of FMDV epitopes is advantageous and can be included in FMDV recombinant vaccine development resulting in vaccines that induce a broad immunological response and thus offer improved protection. In this regard, a bovine immune recombinant phage antibody library was constructed, using tissue samples taken from the (right) prescapular lymph nodes of six Jersey steers vaccinated with a commercial tetravalent FMD vaccine used in Argentina. Following RNA extraction, variable heavy (VH) and light chain (VL) regions of the immunoglobulin G antibody (IgG) genes were amplified by RT-PCR, joined by a flexible linker and cloned into a phagemid vector. The construct was transformed into electro-competent E.coli TG1 cells and an immune library constructed consisting of 1.8 X 107 different clones. Biopanning will be used to identify FMDV-specific single-chain variable fragments (scFvs). These scFvs will be utilised to identify FMDV epitopes and investigate its potential as reagents in a diagnostic ELISA. This is the first report of a FMDV-specific bovine phage library construction.

Poster Session 3 – Monday 20 November

137 - Development of methods to isolate natural porcine antibodies that broadly neutralise porcine reproductive and respiratory syndrome viruses

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Porcine reproductive and respiratory syndrome viruses (PRRSV) cause one of the most economically important diseases of pigs. PRRSV exists as two species, PRRSV-1 and -2, which are both rapidly evolving. The failure of vaccines to provide protection against the diversity of circulating strains poses significant challenges to effective disease control. Passive transfer experiments provide evidence that PRRSV neutralising antibody (nAb) responses are protective. However, PRRSV nAb responses are typically directed against immunodominant variable epitopes and are restricted in breadth. There is therefore a requirement for the development of vaccines that generates a broadly nAb (bnAb) response. We propose that the isolation of monoclonal bnAbs would enable the identification of conserved epitopes, on which broadly protective PRRS vaccines could be designed. To identify donor animals for monoclonal bnAb isolation, we screened sow herds routinely immunised with PRRSV-1 vaccines and which have experienced PRRSV-1 disease breakthroughs. Screening for PRRSV-2 neutralisation in animals with nAbs against PRRSV-1 provides a simple yet effective approach to identify animals with bnAbs. Serum from all 58 sows sampled neutralised PRRSV-1 (titre range 13-12,564) and 67% of the sera cross-neutralised PRRSV-2 (titre range 14-510). To isolate B cells producing bnAbs, we are developing two complimentary approaches. The first uses purified fluorescently tagged PRRSV as an unbiased strategy to label mono- and pan-species specific B cells recognising epitopes on the virion surface. The second approach uses recombinant PRRSV glycoproteins. Recombinant PRRSV-2 GP2, 3, 4 and 5 ectodomains are being expressed in mammalian cells and then assembled as fluorescently labelled tetramers. These strategies will be deployed as 'baits' to facilitate the isolation of single B cells. IgG heavy and light chains will be sequenced and expressed as recombinant monoclonal antibodies. These antibodies would then allow a structural vaccinology approach to the development of broadly protective PRRSV vaccines.

Poster Session 3 – Monday 20 November

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140 - A transcriptional signature of effective vaccination in bovines to Foot-and-Mouth Virus Disease (FMDV)

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Foot-and-mouth disease (FMD) is an extremely contagious viral disease of livestock with high economic impact worldwide. Vaccines have been important for control, but there are serotype specific, and due to high antigenic variation of the virus, are not always fully protective. A deep understanding of the protective immune responses could pave the way for rational design of improved vaccines. In the quest for biomarkers of vaccine-induced protection we evaluated the global transcriptional response after vaccination. Bovines were vaccinated with a commercial FMDV vaccine or remained as unvaccinated controls, and 30 days later whole blood cells were obtained and stimulated in vitro with the vaccine antigen. Individual mRNAs were assessed with a bovine genome-wide microarray. We found 486 differentially expressed genes (DEG) in vaccinated cattle, including 140 up regulated and 346 repressed as compared with naïve animals. Functional enrichment analysis showed that vaccination induced upregulation of genes belonging to functional GO terms associated with inflammatory response and cytokine signaling. In another experiment, bovines were vaccinated as before, and 30 days later challenged with live FMDV. Based on clinical evidence after challenge animals were divided in fully protected and non-protected. Transcriptional responses were assessed just

before the challenge as before. Fully protected animals had 253 up-regulated and 54 down-regulated genes. GO terms linked to protein translation were significantly overrepresented in protected animals. Reactome analysis showed that effective vaccination induced over-expression of 76 metabolic pathways associated with metabolism of RNA and cell cycle as well as some immune system pathways, including "Regulation of IFNg signaling" and "TNF signaling". We selected 59 DEGs for validation with qPCR, several of which allowed us to differentiate successfully vaccinated bovines, from vaccinated and non-protected, and from naïve animals. Results of all these will be presented. Defining biomarkers of vaccine-induced protection shall help development and control of efficient vaccines.

Poster Session 3 – Monday 20 November

142 - Applying phage display screening and next-generation sequencing to identify B-cell epitopes from tick salivary antigens

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Ticks are hematophagous arthropods that affect livestock production and are the main vector of animal diseases. Vaccines are a sustainable alternative for tick control, and multi-antigenic vaccines are considered an option to enhance protection and reduce financial costs. We applied next-generation phage display to identify B-cell epitopes in tick salivary antigens aiming to achieve an epitope-based multi-antigenic vaccine. Hyperimune IgG[OAF1] from cattle immunised with a protective multi-antigenic anti-tick vaccine was submitted to phage display screening using commercial libraries presenting 12-mer linear or 7-mer circularised random peptides. The phages were selected by competitive assay with individual antigens, and next-generation sequencing was performed for peptide identification. The gPhage package was used to deconvolute peptide sequences and frequencies in each library. Peptide enrichment was evaluated by comparing peptide frequencies between libraries derived from immunised and non-immunised animals (Z-score>4). Peptides repeated in two or more libraries were removed, and exclusive peptides from each library were identified. Peptide-coding sequencing found 189,236 and 211,032 linear or constrained peptides selected by the immunised hyperimmune IgG. [OAF2] The similarity within different libraries identified 88% of peptide overlap, resulting in 21,265 and 27,255 library-exclusive peptides. Antigen C had the major number of linear and circularised exclusive peptides, 9,866 and 6,069, respectively, while antigen B had the minor number, four linear and 60 circular peptides. Overall, no significant correlation was identified between the number of peptides selected and protein size, antibody levels, and seroconversion rates. In conclusion, our data show that next-generation phage display can be applied to select potential epitopes screened from hyperimmune sera. The next steps comprise the clustering of peptides for each antigen and mapping the clusters on the surface of the 3D structure to define the most relevant epitopes to comprise a chimeric anti-tick vaccine. FAPESP 2015/09683-9, 2018/23579-8. 2022/07400-3, CAPES, CNPq.

Poster Session 3 – Monday 20 November

165 - A USDA-licensed platform approach for the rapid generation and commercial deployment of bacterial subunit vaccines against emerging diseases

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Rapid deployment of vaccines against emerging and rapidly mutating animal diseases is a challenge for animal agriculture. Although newer technologies accelerate the discovery of vaccines to address new diseases or strains, commercialization and regulatory approval frequently results in a significant lag to market that could previously be solved only with autogenous vaccines. In 2018, the USDA pioneered a new regulatory pathway, termed Prescription-Platform vaccines. Licensed under these guidelines, Medgene has addressed a large number of commercial diseases of the swine and cattle industry and have used this platform to rapidly develop and deploy a highly protective vaccine against a foreign-animal disease outbreak in the United States, Rabbit Hemorrhagic Disease. While current licensees are focused on viral diseases, there is an equally pressing need for bacterial vaccines to provide broad protection against pathogens affecting food-animal species. To that end, we developed a new non-antibiotic based bacterial expression platform to produce vaccines against bacterial pathogens of food animals which is targeted for a similar Platform license. This system was found to efficiently produce antigen in bulk culture and has been tested in final formulations in mice and cattle. These vaccines are tolerated at least as well as currently licensed commercial vaccine formulations and tested for the ability to induce an appropriate serological response indicative of disease protection. To define appropriate bacterial target proteins, we have developed an extensive bioinformatic approach to identify potential immunogenic target proteins from both bacteria and viruses, as well as tools to predict immunogenicity, protein stability, and protein conformation in the final vaccine product. Finally, we have developed unique tools to meet regulatory requirements for vaccine formulation. This approach is a viable, in-practice way to address numerous existing and pending animal diseases in a safe, proven vaccine platform.

Poster Session 3 – Monday 20 November

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197 - Vaccination against the translationally controlled tumor protein (TCTP) of ***Babesia bovis*** reduces clinical disease, improves humoral immune response in cattle, and it is highly conserved in ***Babesia*** species

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Babesiosis is one of the most prevalent diseases in tropical and subtropical areas in the world, where it affects cattle and horses, among other animals. Tumor translationally controlled protein (TCTP) is a secreted protein that prevents the activation and proliferation of B-cells. The aims of this work were to characterize the TCTP of *B. bovis* and *B. bigemina*, to evaluate its effects on vaccinated cattle and to determine the presence of cross-reactive antibodies in other *Babesia* species. The *tctp* gene from different *B. bovis* and *B. bigemina* isolates were cloned and sequenced. A 100% amino acid identity was observed in all sequences obtained for each species. Bioinformatics tools were used to predict the tertiary structure, and to design peptides containing predicted B-cell epitopes. The peptides were used to generate antibodies. Transcription was assessed in mRNA from intraerythrocytic parasites. Expression was confirmed by western blot and confocal microscopy. Subsequently, four *Bos taurus* steers were immunized with the *B. bovis* TCTP-peptides emulsified with adjuvant. Twenty-four days after the last immunization, the animals were challenged with virulent *B. bovis*. Less severe clinical signs were observed in animals immunized with TCTP. A lower amount of total antibodies was observed in the animals of the control group, in comparison with animals immunized with TCTP. An *in vitro* neutralization assay was carried out. Up to 32-24% inhibition was observed in cultures using sera from TCTP-immunized cattle. Finally, *B. bigemina* anti-TCTP antibodies recognized merozoites of *B. ovata*, *B. divergens* and *B. caballi* by confocal microscopy. It is concluded that a) *tctp* is a gene that is expressed in

intraerythrocytic stages, b) TCTP induces neutralizing antibodies, c) *B. bovis* TCTP has a role in the establishment of infection and d) there are cross-reactive anti-TCTP antibodies in *B. bigemina*, *B. ovata*, *B. divergens* and *B. caballi*.

Poster Session 3 – Monday 20 November

201 - Early stages of developing a subunit bacterial vaccine against camel mastitis

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Background: Camel mastitis causes immense economic losses due to diminished milk production and premature culling, thus threatening pastoralists' livelihoods in arid and semi-arid (ASAL) regions of Kenya. The most important cause is bacteria, some of which are zoonotic and thus of public health importance. Due to the complex anatomy of the camel udder, use of the intra-mammary route of antibiotic administration is not recommended. An alternative control method was therefore considered in the face of the current spectre of worsening climate change, associated with prolonged water scarcity for udder hygiene,

Objectives: We aimed to sequence and assemble the genomes of several causative bacteria isolated from camels in various ASAL regions in Kenya and computationally predict putative vaccine candidates for experimental validation.

Methods: Used VaxiJen, IgPred, AllerTOP v.2, DeepLoc and DeepTMHMM, to uncover several potential subunit vaccine candidates and delineated their antigenicity, allergenicity, sub-cellular localisation and transmembrane helices (Table 1).

Results: Computationally delineated potential camel mastitis subunit vaccine candidates are shown in Table 1 (below) obtained from assembled genomes of local Streptococcus agalactiae and Staphylococcus aureus strains.

Table 1: Potential camel mastitis subunit vaccine candidates

GenBank Accession	VaxiJen Score	Subcellular localization	Transmembrane helices	AllerTOP prediction	Signal peptide
MDF3345085.1	1.4003	Extracellular	1	Probable non-allergen	No
MDF3343883.1	0.9351	Extracellular	1	Probable non-allergen	No
MDF3297031.1	1.5055	Extracellular	0	Probable non-allergen	No
MDF3296226.1	1.4155	Cytoplasmic membrane	1	Probable non-allergen	No
MDF3296234.1	1.3336	Cytoplasmic membrane	1	Probable non-allergen	No

Conclusion: We have isolated and sequenced bacterial strains implicated in camel mastitis; and identified an array of putative candidates for a subunit vaccine. Importantly, our work provides a robust platform for further investigations aimed at the development of an efficacious camel mastitis subunit vaccine development.

Poster Session 3 – Monday 20 November

215 - Assembling Newcastle disease virus-like particles in Nicotiana benthamiana plants as potential vaccine

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Newcastle disease (ND) is a highly contagious viral respiratory and neurological disease that has a severe impact on poultry production worldwide. In the present study, an expression platform was established for the transient production in N.bethamiana of Newcastle Disease (ND) virus-like particles (VLPs) for use as vaccines against ND. The expression of the ND Fusion (F) and/or Hemagglutinin-neuraminidase (HN) proteins of a genotype VII.2 strain formed ND VLPs in planta as visualized under the transmission electron microscope, and HN-containing VLPs agglutinated chicken erythrocytes with hemagglutination (HA) titres of up to 13 log2. The immunogenicity of the partially purified ND VLPs was confirmed in specific pathogen-free White leghorn chickens. Birds receiving a single intramuscular immunization with 1024 HA units (10 log2) of the F/HN ND VLPs administered with 20% [v/v] Emulsigen®-P adjuvant, seroconverted after 14 days with F- and HN-specific antibodies at ELISA titres of 5705.17 and HI geometric mean titres (GMTs) of 6.2 log2, respectively. Furthermore, these ND-specific antibodies successfully inhibited viral replication in vitro of two antigenically closely related ND virus isolates, with virus-neutralization test GMTs of 3.47 and 3.4, respectively. Plant-produced ND VLPs have great potential as antigen-matched vaccines for poultry and other avian species that are highly immunogenic, cost-effective, and facilitate prompt updating to ensure improved protection against emerging ND field viruses.

Poster Session 3 – Monday 20 November

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216 - Efficacy of a plant-produced infectious bronchitis virus-like particle vaccine in specific pathogen-free chickens

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Infectious bronchitis (IB) is a highly contagious, acute respiratory disease in chickens, that is listed by the World Organisation for Animal Health (WOAH) and impacts poultry production globally. Its high mutation ability has resulted in numerous variants against which the commercially available live or recombinant vaccines singly offer limited protection. The rapid emergence of regional variants of this Gammacoronavirus warrants new vaccine approaches that are more humane and rapid to produce than the current embryonated chicken egg-based method used for IB variant vaccine propagation (chemically inactivated whole viruses). In this study, IB VLPs were successfully assembled via Agrobacterium-mediated transient expression in Nicotiana benthamiana (tobacco) of a modified full-length IBV spike (S) protein of a QX-like IB variant as potential vaccine candidate. Strong immunogenicity was confirmed in specific pathogen free chickens immunized intramuscularly with VLPs adjuvanted with Emulsigen®-P, where birds that received doses of 5 µg or 20 µg (S protein content) seroconverted after two weeks with mean hemaggluttination inhibition titres of 9.1 and 10 log2, respectively. In a challenge study with the homologous live IB QX-like virus, VLP vaccinated birds produced S protein-specific antibodies comparable to those produced by live-vaccinated birds seroconverting with mean geometric titers of 6.8 and 7.2 log2, respectively. The VLP-vaccinated birds had reduced oropharyngeal and cloacal viral shedding compared to an unvaccinated challenged control and were more protected against tracheal

ciliostasis than the live vaccinated birds. While the results appeared similar, plant-produced IB VLPs are safer, more affordable, easier to produce and update to antigenically match any emerging IB variant, making them a more suitable alternative to IBV control than live-attenuated vaccines.

Poster Session 3 – Monday 20 November

217 - TAHSSL: A new R&D and Commercialization Platform at ILRI

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Research and development (R&D) of vaccines and diagnostics targeting low-and-middle-income countries (LMICs) is disjointed, poorly funded, and viewed as a "high-risk" investment by global and regional animal health companies. As a result, R&D is not optimized for LMIC markets. Challenges include poorly developed challenge models, low return on investment for lengthy and expensive product development, poorly developed LMIC markets, and lack of information on regulatory procedures. ILRI has established the Transforming Animal Health Solutions and Services (TAHSSL) platform to de-risking and accelerate animal health product R&D for LMICs. TAHSSL is a "one-stop-shop" that tests new or improved animal health products by offering quality laboratory, clinical and market development services that meet LMIC market needs while encouraging private sector investments. The platform:

- Designs and implements quality pre-clinical studies
- Evaluates vaccine and diagnostic candidates
- Establishes robust animal disease models
- Helps clients to access market information and authorization

TAHSSL undertakes project-specific partnerships with other LMIC stakeholders in animal health R&D and commercialization. These include GALVmed, ClinGlobal, African Union-PANVAC, regional regulatory authorities, private manufacturing, and distribution companies as well as national and international research institutes. Core funding for TAHSSL is provided by the Bill & Melinda Gates Foundation while project specific funding includes grants from international development funders and project specific investments from the private sector. To date, TAHSSL projects include establishment of a functional world-class clinical research facility undertaking contract research at GLP-like standards (in collaboration with ClinGlobal); establishment of challenge models for CBPP and 5 genotypes of African Swine fever virus; development and evaluation of an integrated CBPP control model consisting of treatment and vaccination; quality assessment of CBPP and CCPP vaccines that are sold in Africa. TAHSSL is open to all potential partners and is ready to support your vaccine and diagnostics R&D and commercialization needs.

Poster Session 3 – Monday 20 November

219 – No longer a regional problem: molecular evolution and transmission of African swine fever on the African continent

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African swine fever (ASF) is a viral haemorrhagic disease of domestic pigs and wild boars. There is neither effective vaccine nor antiviral treatment against ASF.The varied production systems and practices across the pig producing geographical areas in Africa play a key role in the evolution and transmission of ASFV. Previously existing genomic challenges are steadily reducing, opening possibilities for whole genome sequencing of this 185Kb ASFV DNA virus. With this capability, it is now possible to decipher the spatio-temporal dynamics of

ASFV. New genotypes have been recorded in geographical areas where they have not previously existed, while in some instances the existence of genomic alterations with no visible corresponding differences in clinical manifestations has been observed. Finer-scale genomic resolution of the current ASFV genotypes brings in light new approaches in tackling this devastating disease of pigs. The recent developments and approaches could be useful in guiding the ASFV vaccine development strategies in such a way that an individual vaccine could target a group of ASFV genotypes whose distribution is beyond a particular geographical block.

Poster Session 3 – Monday 20 November

223 - Polysaccharide microparticles as carriers of a recombinant antigen against GnRH in oral vaccination in male rats

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Polysaccharide microparticles (PSMs) are a promising carrier system for the oral administration of antigens. They can protect antigens from degradation in the harsh environment of the gut. They can also improve the uptake of antigens by the immune system. Anti-GnRH vaccines work by blocking the binding of GnRH to its receptors on the cells of the hypothalamus and pituitary gland, preventing the release of LH and FSH and consequently suppress sex hormone production. Anti-GnRH injectable vaccines have been developed for a variety of production animals as an alternative to surgical castration. In this work we orally delivered polysaccharide microparticles containing a recombinant fusion protein as an antigen to develop Anti-GnRH response in male rat. We designed and expressed a recombinant 45 KDa peptide (GF45) consisting of a 4 tandem repeats from the GnRH sequence EHWSYGLRPG fused to an immunogenic carrier peptide which contains rickettsial Hsp70 and Hsp60 sequences. GF was encapsulated in alginate-chitosan particles by ionic reverse gelation. Particles containing 200 ug of recombinant peptide were orally administrated to a group of 4-week year old male Sprague-Dowley rats (n=10) in a prime-boost immunization scheme. Empty particles and injectable GF were used as controls. Serum testosterone and anti-GF45 antibodies were analysed by ELISA method along with the weight and volume of testicles. The GF45 orally treated group showed detectable titters of anti-GF45 antibodies and a 95% reduction of circulating testosterone in comparison to the control group. Testicles in treated group showed a reduction of 52% in weight and 50% in volume regarding to control.

The encapsulation and oral delivery of the recombinant GF45 in alginate-chitosan microparticles generated autoimmune response capable of blocking the function of endogen GnRH. These particles are a suitable not only for generating non injectable or surgical control of GnRH but also to possibly deliver other recombinant antigens.

Poster Session 3 – Monday 20 November

224 - Validation of a Modified Live Virus (MLV) prototype of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) with replication-competent expression of porcine interferons (IFNs)

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Background: Porcine reproductive and respiratory syndrome (PRRS) is a complex and costly disease in the swine industry, due in part to the high degree of genetic variation among PRRS virus (PRRSV) field isolates. Although there are PRRSV vaccines currently available, they can have varying degrees of cross-protection depending on genetic similarity.

Objectives: We have identified several host interferons that have superior antiviral properties potentiating immune responses in pigs. Preliminary evaluation of selected interferons (IFNs) was demonstrated in vitro through reverse genetic incorporation into the Type 2 PRRSV p129 vaccine backbone (Zoetis). We performed in vivo studies in pigs to compare novel vaccine candidates to commercially available MLV vaccines using a contemporary challenge virus (NADC-34).

Methods: The animal study was conducted in commercial pigs (n = 10/group): sham vaccine + sham challenge, sham vaccine + challenge, MLV-commercial + challenge, MLV-PRRSVp129-IFNϖ + challenge, MLV-PRRSVp129-IFNmix + challenge. Pigs in all treatment groups were monitored for clinical signs, weighed, and temperature recorded throughout the study. Serum was collected to evaluate viral load with real-time-RT-PCR and the immune response with a commercial PRRSV ELISA, and whole blood was used to evaluate gene expression.

Results: The pilot study demonstrated that antiviral IFN vaccine prototype efficacy was comparable to commercially available PRRSV MLV vaccine. In this study, pigs administered the novel vaccines had similar ELISA titers prior to challenge and reduction in viral load in the serum after challenge to those given the commercial MLV. In addition, the MLV-PRRSVp129-IFNmix numerically reduced temperature and viral load greater than MLV-PRRSVp129-IFNϖ.

Conclusion: A DNA-launched reverse genetics system for PRRSV and co-expression of immunomodulatory peptides designed to directly reverse PRRSV suppression on the pig's IFN signaling and associated immune response has the potential to enhance vaccine efficacy against heterologous PRRSV strains compared to currently available vaccines.

Poster Session 3 – Monday 20 November

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229 - Protective efficacy evaluation of four inactivated commercial vaccines against low pathogenic avian influenza H9N2 virus under experimental conditions in broiler chickens

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AI vaccines are used in the poultry industry. The objective of this study was to compare, the protective efficacy of four imported inactivated H9N2 vaccines (A, B, C, and D) in broiler. A total of 150 one-day-old chicks were divided into 6 groups: 4 experimental groups, each containing 30 chicks, received one of the vaccines (A, B, C, or D) delivered in a 0.3-ml dose subcutaneously at 1 day of age, whereas the control, Group T, was not vaccinated but challenged and Group E was kept unvaccinated and unchallenged. At 21 days postvaccination, Groups A, B, C, D, and T were challenged with 107 embryo infective dose 50% of A/Chicken/Morocco/01/2016 (H9N2). All chicks were observed daily for clinical signs during the 12 days post challenge. At 5 and 12 dpc, chicks were euthanatized for necropsy. Blood samples were collected weekly for serology and oropharyngeal swabs were collected for virus detection by rt-RT-PCR. Respiratory signs started at 48 hr pc and maximum severity was observed on 9 dpc. Chiefly, the birds vaccinated with vaccine B showed significantly more respiratory signs than did their counterparts. Serologic analysis revealed that the sera of Groups A and D birds

showed a decrease in antibody (Ab) levels up to day 26; then a slight increase of Ab level was observed until day 31, while Group B and C birds showed a stabilization of the titers from day 21 until the end of the experiment. The viral shedding rate was lower in Groups C and A (40%–50% of the birds shed virus for ,7 days) compared with other challenged groups (60%–75% of the birds shed virus for 9 days).This experiment illustrated that vaccination applied on the first day in the hatchery with the four vaccines tested did not provide an acceptable protection against H9N2 in comparison with the controls that did not receive any vaccine. At first glance, we might favour vaccines A and C for their ability to reduce and shorten viral shedding as compared with vaccines B and D.

Poster Session 3 – Monday 20 November

Theme 9. Immune-regulation and modulation: Role of mibrobiome

069 - Investigating the soil mycobacteriome to validate M. bovis tuberculin skin test results

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The environment (soil and water) contains a diverse mycobacteriome which can influence mycobacterial infection of hosts and their immune responses. The soil mycobacteriome is composed primarily of non-tuberculous mycobacteria (NTMs) which are saprophytes or opportunistic pathogens. Pathogenic Mycobacteria tuberculosis complex (MTBC) including M. bovis may also be present in the environment if shed by infected hosts. NTMs can elicit a host immune response which may result in cross-reactions to shared antigens of MTBC. Since immunological tests are widely used for the detection of M. bovis infected animals in South Africa, false positive results in high value animals, such as African buffalo (Syncerus caffer), can result in unnecessary economic and genetic losses. Therefore, it is important to investigate suspect TB diagnostic test results and identify NTMs in the environment that may lead to immune sensitization. In this pilot study, the diversity of Mycobacteria spp. within the soil microbiome was evaluated using molecular techniques in an area where suspect tuberculin skin test reactor buffaloes have been reported (M. bovis historically free area). The DNA was extracted from mycobacterial cultures of environmental samples, followed by PCR (rpoB and hsp65) amplification and Sanger sequencing to provide species level identification. In 31.8% (7/22) of the samples, M. bovis was present along with several NTM species. This indicated that although some of the NTMs detected may result in cross-reactivity, the presence of environmental M. bovis was a more likely cause of immune sensitization, reflected by a positive tuberculin skin test result. The molecular techniques used in this study could successfully investigate the soil mycobacteriome and would be recommended for investigating future instances of suspect tuberculin skin test reactor buffaloes.

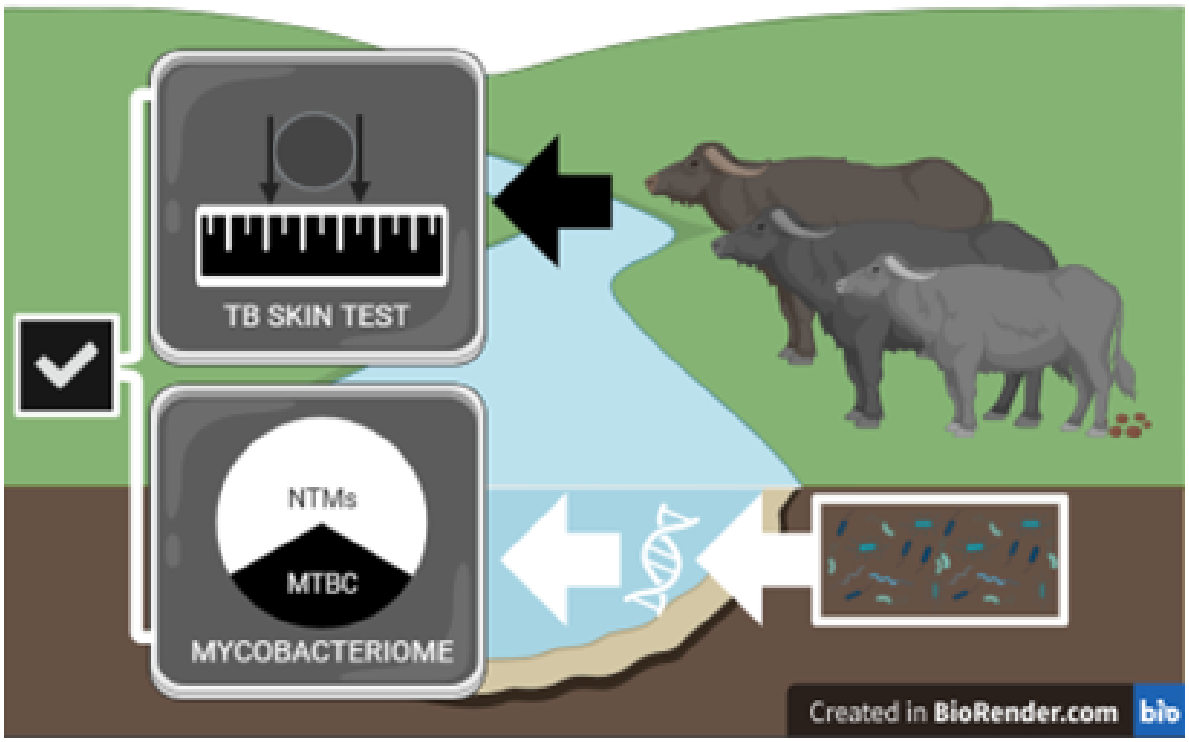

Figure 1. Summary figure: investigating the mycobacteriome with molecular techniques was used to validate TB skin test results from African buffalo.

Poster Session 3 – Monday 20 November

Theme 10. Comparative Immunology

004 - Cannabinoid receptor 2 evolutionary gene loss makes parrots more susceptible to neuroinflammation

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Parrots (Psittaciformes) are birds with advanced cognitive abilities outperforming mammals of similar size. In their evolution they came through massive chromosomal rearrangements, which caused several gene losses. Here we provide genomic evidence of cannabinoid receptor 2 (CNR2) gene loss shared in parrots. Our results based on interspecific comparison of immune response regulation in parrots (CNR2 loss) and passerine birds (functional CNR2) suggests susceptibility of parrots to neurinflammation. In budgerigar (Melopsittacus undulatus), we detected a significant upregulation of proinflammatory cytokines including interleukin 1 beta (IL1B) and interleukin 6 (IL6) expression in the brain after experimentally induced sterile peripheral inflammation. In contrast with the parrots, no such upregulation was detected in zebra finch (Taeniopygia guttata). We propose that CNR2 loss, which acts as an immune regulator expressed mainly in immune cells including microglias in brain, might have contributed to parrot susceptibility to neurological disorders like depression-related behaviours. For this purpose, parrots may serve as suitable models in inflammation-behaviour neurological studies.

033 - Exploratory screening for miRNA biomarkers in canine multicentric lymphoma

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Background: Lymphoma is one of the most frequent hematopoietic tumors in dogs and shares similar features with human counterparts. MicroRNAs (miRNA, small non-coding RNAs) are pivotal in gene regulation fine tuning and cancer hallmarks are influenced by their aberrant expression. Consequently, miRNA biomarkers may assist predicting therapeutic response and clinical outcome by providing less-invasive novel diagnostics tools. The aim of this study was to detect dysregulated miRNAs in lymphomatous lymph node tissues in comparison to lymph node material or PBMCs from healthy control dogs. Potential significant differences in miRNA expression profiles between four lymphoma entities were evaluated.

Methods: A customized PCR array was utilized to profile 89 canine target miRNAs. Quantification was performed using quantitative real time PCR and relative expression was determined by the delta-delta Ct method using the GeneGlobe Data Analysis Center (Qiagen, www.qiagen.com). P-values were calculated by using the student's t-test and p-values less than 0.05 were considered statistically significant.

Results: In 28 canine samples, 85 out of 89 miRNAs were successfully amplified, showing many being differentially expressed in the lymphoma entities. In the 14 diffuse large B-cell lymphoma (DLBCL) patients, 28 and 24 different miRNAs were significantly dysregulated compared to lymph node material or PBMCs. Sixteen miRNAs occurred in both control groups, with 12 miRNAs being down- and 4 miRNAs being upregulated. The six peripheral T-cell lymphoma (PTCL) samples showed 24 and 25 dysregulated miRNAs when compared to the healthy controls. A combined analysis of DLBCL and PTCL samples revealed seven shared and 19 differently expressed miRNAs.

Conclusions: Potential biomarkers in T- and B-cell lymphoma could be the miRNA-17-92 cluster, and miRNA-181-family together with miRNA-34a and miRNA-150. A panel of 26 significantly dysregulated miRNAs will be applied to confirm and validate these miRNAs together with those with unknown function and still missing literature record.

Poster Session 3 – Monday 20 November

166 - Effects of hydrocortisone, ascorbic acid, and thiamine treatment on immune responses in healthy neonatal foals

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Sepsis is a leading cause of morbidity and mortality in neonatal foals and induces oxidative and inflammatory dysregulation. Combination therapy with low-dose hydrocortisone, ascorbic acid, and thiamine (HAT) may offer an affordable immunomodulatory approach in equine sepsis, but effects of HAT in foals are unknown. We hypothesized HAT treatment in healthy foals would modulate bacteria-induced oxidant and inflammatory cytokine responses. Healthy 2-day-old foals were randomly assigned to receive HAT (1.3 mg/kg/day hydrocortisone, 400 mg/kg/day vitamin C, 20 mg/kg/day thiamine, n = 8) or saline placebo (n = 8) intravenously

q.6h for three days. Plasma antioxidant capacity (PAC) and reactive oxygen metabolites (d-ROMs) were measured via a validated photometric assay before, 48 hours into, and 48 hours after treatment. Peripheral blood leukocytes were collected at these time points and stimulated ex vivo with killed whole-cell *Staphylococcus aureus*, *Escherichia coli,* or PBS (control) for 6 hours, after which d-ROMS were measured as above, and bacteria-induced inflammatory cytokine (TNFa, IL-1b, IL-6, IL-10) production was quantified via ELISA. Groups were compared via mixed-effects modeling with Holm-Šidák post hoc analysis and with unpaired t-tests ($p<0.05$). Plasma d-ROMs were similar between and within groups at baseline and after cessation of treatment but were decreased during HAT treatment in treated foals compared to controls ($p\leq0.002$). *E. coli*-induced ex vivo dROM production was also decreased during HAT treatment ($P=0.005$). There was a significant interaction ($P<0.048$) between treatment (HAT/placebo) and time (before, during and after treatment) on ex vivo IL-6 and TNFa production, and a trend towards decreased bacteria-induced IL-6 production during HAT treatment ($P<0.051$). Three days of HAT treatment in healthy foals decreased circulating oxidant metabolites and modulated bacteria-induced ex vivo immune responses. Further study is needed to determine if HAT therapy offers beneficial immunomodulatory effects in equine sepsis.

Poster Session 3 – Monday 20 November

170 - MDP-mediated chronic NOD2 stimulation confers protection against LPS challenge through M2b macrophage polarization in mice

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Innate immune cells show enhanced responsiveness to secondary challenges after an initial non-related stimulation (termed Trained Innate Immunity, TII). Acute NOD2 activation by Muramyl-Dipeptides (MDPs) promote TII inducing the secretion of pro-inflammatory mediators, while a sustained (chronic) MDP-stimulation can promote NOD2 degradation and down-regulate the inflammatory response, restoring both cross and self-tolerance. Chronic NOD2 stimulation has not been deeply explored although peripheral macrophages are continuously exposed to MDPs from microflora breakdown. Here we characterized in-vitro MDP-trained murine macrophages upon an experimental challenge with high concentrations of LPS in the context of chronic inflammation induced by a sustained stimulation of NOD2. Raw264.7 cells were trained with MDP (1µg/ml, 48h) and challenged with LPS (5µg/ml, 24h). Each determination was assessed in three experiments, three replicates each. ANOVA test was assessed, followed by Tukey Test for comparisons between treatments. Trained cells formed multinucleated giant cells with increased phagocytosis rates compared to untrained/challenged cells. They showed a reduced metabolic activity with evidence of a switch to aerobic glycolysis. LPS upregulated TNFα and NO with similar levels in both cultures ($p>0.05$) while IL10 was upregulated and IL12 downregulated in trained cells ($p<0.05$). MHCII and B7.2 expression were significantly upregulated in the trained compared to control cells ($p<0.05$). A protective effect was observed when assessing the survival rate of trained cells under nitrosative stress compared to untrained/challenged cells ($p<0.01$). The relative expression of PARP-1 (which inhibition triggers TII) was downregulated after the LPS challenge, which may contribute to the regulatory milieu and to the innate memory mechanisms exhibited by MDP-trained cells. Our results demonstrate that a sustained MDP-training polarizes murine macrophages towards a M2b profile. PARP-1 downregulation may confer resistance to LPS challenge even under stressful conditions. These results impact the development of immunomodulatory strategies to control processes in which inflammation should be restored.

Theme 11. Zoonoses

139 - *Mycobacterium tuberculosis* in a Captive African Elephant: Identification of Mixed Infection Using Whole Genome Sequence Data

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Zoonotic and reverse zoonotic tuberculosis pose a risk to human and animal health, especially when individuals are in close contact. The recent innovation of whole genome sequencing (WGS) has led to significant advancements in our comprehension of bacterial disease dynamics, particularly regarding the transmission of pathogens at the population and individual levels. *Mycobacterium tuberculosis* was cultured from respiratory samples, including ante-mortem trunk wash, bronchoalveolar lavage, and post-mortem lung tissue samples of one captive elephant euthanized in a South African zoo. The elephant presented with chronic weight loss and lethargy. Animal-side serological testing (Chembio DPP® VetTB for Elephants) conducted on elephant serum yielded a positive result before euthanasia. At post-mortem examination, signs of chronic pneumonia and extensive macroscopic lesions compatible with tuberculosis were observed, confirming the presence of disease. A total of four crude liquid culture MGIT extracts were subjected to WGS analysis (Illumina paired-end sequencing). Interestingly, the study identified a mixed infection involving two distinct strains of *M. tuberculosis*. The predominant strain was classified as Lineage 1 and a second strain was identified as Lineage 4. Both lineages have been found in a significant proportion of human tuberculosis cases in this country. No mutations associated with drug resistance were detected. The report highlights the susceptibility of elephants to human pathogens, particularly in high-burden settings. Biosafety challenges associated with handling and diagnosing tuberculosis in captive elephants are reported. We emphasize the importance of implementing effective preventive measures to ensure the safety of both humans and animals in zoo environments. Finally, the importance of multiple sampling and analysis of within-host mycobacterial populations for investigations of transmission is demonstrated.

Poster Session 1 – Saturday 18 November

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164 - Saliva as a non-invasive tool for monitoring microbial diversity and pathogens in wildlife

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One Health unites human, animal, and environmental health in a multidisciplinary approach, proven crucial during recent outbreaks of emerging diseases (*e.g.,* SARS-CoV-2 pandemics). Studying pathogens' prevalence and health status of wildlife is essential, as these directly affect public health. The development of effective and non-invasive sampling methods is urgent. Predictive models can only be achieved through large databases but current invasive sampling methodologies involving animal chemical/mechanical immobilization hinders

our ability to generate data at a landscape scale. Saliva has been gaining traction in recent years as an invaluable source of information for monitoring health, welfare, and pathogen circulation. The present study aimed to determine whether wildlife saliva samples are compatible with microbial and health monitoring. Saliva samples from different species, including capybaras and marmosets, were stored under different environmental conditions (temperature, relative humidity, and length of storage) to mimic working field conditions with no available cold chain. At predetermined time points, the samples were assayed for DNA/RNA isolation and quality control, pathogen-specific RT-qPCR, and 16S metagenomics sequencing. So far, our results showed that DNA remains stable up to 60 days after collection, allowing detection of specific pathogens by RT-qPCR, and generating relevant information for microbial discovery through deep sequencing. We found that composition of bacterial communities in the capybara's saliva is atypical among all other mammals studied so far. Our results reinforce the recent attention given to saliva samples as a robust, easily accessible, and informative medium for acquisition of data on health markers, including immunity status, and call for additional efforts in adjusting molecular techniques for the addition of this useful medium to the veterinary immunologist's toolbox.

Poster Session 1 – Saturday 18 November

168 - The use of interferon-gamma releasing assays (IGRA) to improve the detection of tuberculosis in captive-bred non-human primates

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Tuberculosis (TB) caused by the *Mycobacterium tuberculosis* complex (MTC) continues to be an important zoonotic disease in captive non-human primates (NHPs) in biomedical facilities. Detection of MTC in NHP colonies is routinely done by tuberculin skin test (TST) whose readouts are subjective and liable to false-negative interpretations. Therefore, there is a need to develop and optimize a laboratory-based method to supplement TST in MTC detection. This study explored the use of the available interferon-gamma (IFN-γ) release assays to identify a suitable one for MTC detection in African green monkeys (AGMs). Blood samples were obtained from 45 AGMs during routine TST screening and suspected MTC cases for detection of TB using the QuantiFERON-TB Gold Plus (QFT) and TB ELISpot tests. Freshly isolated peripheral blood cells were used in the ELISpot assay while whole blood was used in QFT tests. The samples used in QFT tests were further divided into three groups in which the positive controls were supplemented with either 5 µg/ml or 2 µg/ml of concanavalin A (con A) mitogens or without supplementation during the 37°C overnight incubation and the IFN-γ was quantified in the harvested plasma using two commercial ELISA kits. The QFT identified 6 of 45 samples as TB-positive and the supplementation with 5µg/ml significantly increased the quantities of IFN-γ in the positive controls for both kits. Seven of 45 samples tested TB-positive in the TB ELISpot assays while another 5 samples were only positive for *Bacillus Calmette-Guerin* (BCG) antigens. All animals testing positive for TB and BCG antigens were confirmed TB-infected at post-mortem. The data showed that supplementing the positive controls with con A potentially improved the quality assurance in QFT assays whilst TB ELISpot assays can be used in conjunction with TST to improve TB screening in captive NHPs.

Poster Session 1 – Saturday 18 November

195 - Isolation of bacteriophages against salmonella isolates from environmental water samples and gorilla feces collected from Bwindi Impenetrable National Park

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Salmonella spp is considered an important cause of enteric disease in gorillas and also a public health threat. There are increased risks of highly pathogenic antimicrobial resistant bacterial pathogens between humans and the wildlife due to increasing human activity in form of tourism and research. This study aimed at establishing a stock of bacteriophages against Salmonella spp isolates from Gorillas from Bwindi Impenetrable National park (BINP) as an insight for future exploration of bacteriophage therapy in both animals and humans to combat antimicrobial resistance. A cross sectional study was carried out between November, 2017 and February 2018, and a total of 93 samples (62 fecal and 30 stream water samples) were collected from Bwindi Impenetrable National Park for isolation of Salmonella; while for bacteriophages, sewage samples from the National Water and Sewage Corporation (NWSC) plant were also obtained. Salmonella spp was isolated by culturing on Xylose Lysine Deoxycholate (OXOID UK) and then confirmed by biochemical tests. Out of the 62 gorilla fecal samples, only two tested positive for Salmonella (3.2% prevalence), obtained from Habinyanja group; whereas none were obtained from the water. Bacteriophages against Salmonella isolates were obtained from the sewage samples and none was found water from the park. The bacteriophages exhibited no activity against Salmonella stock cultures earlier isolated from reptiles; Crocodylus niloticus (Nile crocodile), Bitis gabonica (Gaboon viper), Stigmochelys pardalis (Leopard tortoise) and Leptoptilos crumenifer (Marabou stork). The plaque forming titer value of the two Salmonella bacteriophages was 7.0×108 pfu ml-1 and 7.0×1011 pfu ml-1, respectively. Sensitivity to phages by the Salmonella spp. isolates creates hope for developing future alternatives to conventional antibiotic therapy which is currently challenged by antimicrobial resistance. There is need for further characterization of the bacteriophage isolates to determine the genotype, and sequencing to establish absence of undesirable genes.

Poster Session 1 – Saturday 18 November

Theme 12. Immunoinformatics

026 - ***In silico*** prediction of CD4⁺ T cell and B cell epitopes targeting ***H. contortus*** vaccine antigens

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Haemonchus contortus is a pathogenic nematode affecting livestock production globally, especially in tropical areas. Anthelmintic drugs are used to treat *H. contortus* infection, however their use has resulted in drug resistance. There is a vaccine that can provide protection, but multiple vaccinations and boosters are required to achieve the desired immunity, thus highlighting the need for alternative vaccines. This vaccine is made up of native gut proteins, H11 and *Haemonchus* galactose containing glycoprotein (H-Gal-GP) complex composed of aspartyl proteases, metalloproteases and cysteine proteases. These proteins have not been explored for prediction of epitopes that can be used as potential vaccine candidates in multi-epitope vaccines. Hence our study aimed at using an immunoinformatic approach to predict CD4⁺ T cell and B cell epitopes of

the H11 and H-Gal-GP complex. Several immunoinformatic tools were used to identify conserved $CD4^+$ T cell and linear B cell epitopes that were predicted to be antigenic, non-allergic and non-toxic. Additionally, the ability of $CD4^+$ T cell epitopes to induce IL-4, IFN-γ and IL-10 was further predicted *in silico*. Nine $CD4^+$ T cell epitopes and seven linear B cell epitopes were predicted from H11, while five $CD4^+$ T cell epitopes and four linear B cell epitopes were predicted from the cysteine proteases. Additionally, three $CD4^+$ T cell epitopes and three B cell epitopes were predicted from metalloproteases while two $CD4^+$ T cell epitopes and one B cell epitope were predicted from Aspartyl protease. All the epitopes were predicted to be highly antigenic, probable non-allergens, and non-toxins while all the $CD4^+$ T cell epitopes were predicted to induce IL-4 only. The identification of these epitopes provides the basis for further *in vitro* testing to evaluate their ability to induce an immune response.

Poster Session 1 – Saturday 18 November

114 - ***In silico*** prediction of CD4 T cell and B cell epitopes of the peptidase and GTPase protein families of ***Haemonchus contortus***

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Haemonchus contortus, also known as "Barber's pole worm", is one of the world's most pathogenic parasitic nematode that infects small ruminants such as sheep, goats in subtropical and tropical regions globally. The current global anthelmintic drug resistance together with the shortfalls of the available commercial vaccine, Barbervax®, emphasise the need for alternative control measures such as new vaccines for *H. contortus*. In an attempt to work towards the design of an alternative vaccine against *H. contortus*, an immunoinformatic approach was applied to predict CD4 T cell and B cell epitopes of peptidase and GTPase proteins of *H. contortus*. The peptidase and GTPase protein sequences of *H. contortus* were retrieved from NCBI and analysed using various immunoinformatic tools to predict only conserved CD4 T cell and B cell epitopes that were antigenic, non-allergens, and non-toxic. Thereafter the ability of the CD4 T cell epitopes to induce the cytokines IL-4, IL-10 and IFN-Ɣ were also predicted using immunoinformatic tools. Ten CD4 T cell epitopes predicted to induce IL-4 only and four conserved linear B cell epitopes from the GTPase protein family that were antigenic, non-allergenic and non-toxic were identified. The *in silico* prediction and identification of CD4 T cell and B cell epitopes provide a basis for further *in vitro* immunogenicity testing.

Poster Session 1 – Saturday 18 November

186 - ***In silico*** analysis of GPI-anchored hypothetical proteins from Babesia bovis as potential vaccine candidates

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Babesia bovis is a major impediment to livestock production in tropical areas including South Africa. Control measures against this parasite include the use of acaricides, chemotherapeutic drugs, and live attenuated vaccines, which are limited in their efficacy. Recombinant vaccines have been shown to provide protection in cattle against *B. divergens* infection. Similarly, dogs could be protected against *B. canis* infection by immunization with the homologous recombinant protein of *B. canis*. Pathogenic protozoan parasites contain glycosylphosphatidylinositol (GPI) anchored proteins, which are considered primary candidates for vaccine development. Using bioinformatics tools, 17 proteins with predicted GPI anchors were identified from the proteome of *B. bovis*. Five belong to the previously characterized variable merozoite surface antigen (VMSA) family and one, GASA-1, was recently characterized. In this study, in silico approaches were used to analyze the 11 uncharacterized GPI-anchored proteins. The *B. bovis* GPI-anchored hypothetical protein sequences were retrieved from the UniProt database and subjected to analysis for signal peptide sequences (SignalP 6.0), GPI-anchor site sequences (GPI-SOM, PredGPI), and C-terminal transmembrane domains (DAS, Topcons and TMHMM). Transmembrane sequences were identified using the SOSUI server. The antigenicity was predicted using VaxiJen 2.0 and B and T cell epitopes were identified using the method of Kolaskar & Tongaonkar, IEDB MHC-I, and NetMHCII pan tools. The physicochemical properties were assessed using ExPASy - ProtParam tool. Our analysis confirmed optimistic GPI-anchor prediction for the 11 hypothetical proteins, which also contained N- and C-terminal transmembrane domains. The overall antigen prediction score was above the VaxiJen 2.0 threshold of the 0.5 parasite model, indicating probable antigenicity for 7 of the 11 hypothetical proteins analyzed. Predicted epitopes with strong binding to bovine leukocyte antigens (BoLA) molecules of MHC classes were also identified. Based on the analysis, three hypothetical proteins were selected for further characterization.

Poster Session 1 – Saturday 18 November

Theme 13. Immunology of wildlife and exotics

035 - Cytokine Release Assay for the Detection of ***Mycobacterium bovis*** Infection in African Lions (***Panthera leo***), Cheetahs (***Acinonyx jubatus***) and Leopards (***Panthera pardus***)

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Bovine tuberculosis (bTB) is a chronic disease caused by *Mycobacterium bovis*. In South Africa, bTB affects many of the country's most iconic wildlife species including leopards (*Panthera pardus*), lions (*Panthera leo*), and cheetahs (*Acinonyx jubatus*), which poses a risk to ecotourism and conservation. The aims of this study were to identify commercially available feline cytokine ELISAs that have the potential to detect lion, cheetah and leopard cytokines and develop an antigen-specific cytokine release assay (CRA) that can distinguish between *M. bovis*-infected and uninfected wild felids. Commercially available feline cytokine ELISAs for TNF-α, IL-1β, and IFN-γ were screened for the quantification of cytokine proteins in plasma from lion, cheetah, and leopard whole blood stimulated using QuantiFERON®-TB Gold Plus (QFT) tubes. The optimal feline ELISA was chosen as the assay that showed reactivity between antibodies and felid protein cytokines and was able to distinguish between *M. bovis*-infected and uninfected lion, cheetah, or leopard. High mitogen responses were observed in all three assays using samples from all three species. However, the Mabtech Cat IFN-γ ELISA^{Basic} was the only kit that could distinguish between *M. bovis*-infected cheetah (IFN-γ concentration 738 pg/ml), lions (up to 1074 pg/ml) and leopards (up to 3790 pg/ml), from uninfected individuals (0 pg/ml) in each species. In addition, preliminary IGRA cut-off values were calculated for cheetah (11 pg/ml), leopards (14 pg/ml), and lions (33 pg/ml). The CRA was partially validated for use in lions with good parallelism, linearity, and minimal matrix interference. These preliminary results suggest that the QFT-Mabtech Cat IFN-γ release assay has potential as a possible bTB diagnostic test for wild felid species. The development and validation of CRAs for wild felids will provide the basis for screening translocation candidates and facilitate conservation programmes in southern Africa.

Poster Session 1 – Saturday 18 November

158 - Dexamethasone increases in vitro immune cell proliferation in response to M. bovis in African buffalo

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Mycobacterium bovis, the causative agent of bovine tuberculosis (bTB) in livestock and wildlife, is a zoonotic disease responsible for over 12,000 human deaths annually. In sub-Saharan Africa, the African buffalo (*Syncerus caffer*) acts as the primary maintenance host for bTB in the wild. Progression of bTB in buffalo is dependent on the animal's immune response, which can be weakened by stress-related events, triggering the release of glucocorticoids, known to suppress the immune system. Dexamethasone (DEX), a synthetic glucocorticoid, has been used experimentally as a model of stress-induced immunosuppression in cattle and has been associated with decreased lymphocyte proliferation to mitogens. In this study, we investigated the effects of DEX on the proliferation of peripheral mononuclear cells (PBMCs) of wild African buffalo, under varied in vitro antigenic stimulations, including bTB antigens. We isolated PBMCs from 13 wild-caught buffalo and stimulated them with pokeweed mitogen (PKW) and tuberculin purified protein derivative (PPD) in both the presence and absence of DEX. We also stimulated the buffalo PBMCs with a protein antigen derived from *Haemonchus contortus*, a common parasite in African buffalo, to investigate whether the effects of DEX are antigen-specific. We found that DEX significantly enhanced proliferation in 60% of the buffalo PBMC samples in the presence of PPD when compared to PPD and PKW alone or PKW in the presence of DEX. However, DEX did not significantly increase PBMC proliferation in the presence of the *Haemonchus contortus* antigen. These results suggest that immune cell proliferation in response to M. bovis is enhanced in the presence of DEX. This in vitro study suggests new avenues for further investigation into the molecular mechanisms of corticosteroid action on immune cells within the specific context of tuberculosis. These findings provide novel insights that can contribute to the development of host-directed therapies in humans and livestock.

Poster Session 1 – Saturday 18 November

184 - Strengths and Opportunities of One Health Approach for Rabies Control in the Jhalakhati District, Bangladesh, 2022

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Background: Rabies prevention and control requires multi-sectoral collaboration. Jhalakhati is a district of Barishal division of Bangladesh. From July to December 2022, Jhalakhati reported 56 positive rabies dog heads to the national animal rabies surveillance, top ranking in Bangladesh. This study aimed to describe and evaluate One Health rabies prevention and control practices, both routine and during outbreak periods.

Methods: Stakeholders in animal, human, and local administrative sectors were interviewed under National Rabies Elimination Program of Directorate General of Health Services (DGHS) in the Jhalakhati district rabies prevention and control practices of Mass Dog Vaccination (MDV). Document reviews and observations on routine rabies prevention and control practices were also performed.

Results: Health and veterinary sectors under Jhalakhati district authority provide rabies control programs (i.e., health literacy, vaccination campaign, sterilization of stray dogs, and shelter support). However, the survey of dog populations was not implemented. Managing stray dogs is difficult. During animal rabies event occurrence, both health and veterinary sectors have collaborated. Contact tracing and vaccination among rabies animal bite patients were performed. Also, animal ring vaccination was done; however, only 200 meters of radian coverage area instead of five kilometers as a guideline.

Conclusions: Jhalakhati district has ability, resources and facilities to implement the One Health approach for rabies prevention and control. However, they still need aid and guidance from central government sectors. The health sector should promote pre-exposure prophylaxis vaccination campaigns among people in the rabies events area. Boom vaccination among susceptible rabies animals with assistance from the Department of Livestock Services should be considered. In addition, Jhalakhati district should do an active survey of the dog population to estimate the coverage of the rabies prevention program.

Poster Session 1 – Saturday 18 November

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213 - Proteogenomic analysis reveals lncRNA-encoded immunopeptides in the devil facial tumour disease

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The devil facial tumour disease (DFTD) was the primary reason for the listing of Tasmanian devils (Sarcophilus harrisii) as an endangered species. The disease is caused by two independent devil facial tumours (DFT1 and DFT2) that are transmitted among wild devils via biting. We have previously confirmed that a human adenovirus can transduce Tasmanian devil cells and potentially serve as a vaccine platform. For the proposed adenovirus-based vaccine to be effective, multiple tumour-specific antigens need to be identified and encoded into the vaccine. Proteogenomic studies in multiple human cancers have revealed that many putative non-coding regions are aberrantly expressed and translated into proteins and thus represent a major source of tumour-specific antigens. Our goal was to identify putative long non-coding RNA (lncRNA) that are aberrantly expressed and potentially translated into proteins in DFT1 and DFT2. We used bioinformatics tools to analyse multiple RNAseq datasets from DFT1 and DFT2 for identification of lncRNAs with protein-coding potential. Using proteogenomic methods, we compared the lncRNA-derived proteins to mass spectrometry data from DFT1 and DFT2, to confirm expression of the identified lncRNA at protein level. The lncRNA-encoded proteins were searched against DFT1 and DFT2 immunopeptidomes, to determine whether they are processed into potentially targetable MHC-I peptides. We identified twenty-eight peptides containing 8-16 amino acid residues that were presented on DFT1 and/or DFT2 MHC-I but not on devil fibroblast MHC-I. Seventeen of these peptides were found in DFT1, eight peptides were found in DFT2, and three peptides were found in both DFT1 and DFT2. The discovery of DFT1- and DFT2-specific MHC-I peptides that originate from non-coding regions expands the pool of target antigens for the proposed DFTD vaccine.

Poster Session 1 – Saturday 18 November

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226 - Vaccination of African Penguins (Spheniscus demersus) against H5 high pathogenicity avian influenza: a comparison between an inactivated whole-virus vaccine and a plant-produced virus-like particle

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High pathogenicity avian influenza (HPAI) has become a conservation threat to wild birds worldwide, besides being a danger to domestic poultry. African Penguins (Spheniscus demersus) have been among the endangered seabird species affected by the disease in Southern Africa, since 2018. However, options to reduce the impact of outbreaks are very limited. Conventional whole-virus inactivated vaccines are slow to produce and to adapt to circulating field strains. They also require parenteral administration and at least two doses, so their application to wild birds seems unrealistic. Suitable vaccine technology and practical application methods therefore require investigation. Twenty-four captive African Penguins were vaccinated with either a conventional inactivated clade 2.3.4.4b H5N8 HPAI whole virus or a tobacco leaf-produced H5 virus-like particle (VLP). Six birds received a second dose of the inactivated vaccine, 56 days after the initial dose. The magnitude and duration of serum antibody responses were assessed and compared, employing haemagglutination inhibition tests. Bacterial contamination of the VLP vaccine limited the monitoring period and sample size in that treatment group. However, it could be established that the second dose of inactivated vaccine was required to induce antibody titres above the level required to suppress virus shedding (≥128) while a single dose of VLP vaccine produced a geometric mean titre of 424 by day 14. One bird in the VLP

groups still tested positive (titre = 16) on day 430 while negative titres were reached in the once- and -twice-vaccinated inactivated vaccine groups by days 84 and 175 respectively. VLP vaccines offer a more practical option than inactivated whole viruses, especially in logistically challenging situations involving wild birds. Further investigation is required to confirm the duration of antibody response after a single dose, and to explore the possibility of mucosal administration, to make vaccine application more feasible in the field.

Poster Session 1 – Saturday 18 November

Theme 14. Immunogenomics and resistance to disease

131 - Continent-wide population genomics of the African buffalo (***Syncerus caffer***) indicates infectious disease as a significant selective pressure

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The African buffalo (*Syncerus caffer*) is a wild bovid with a historical distribution across much of sub-Saharan Africa. Genomic analysis has the potential to provide insights into the evolutionary history of the species, and potentially the key selective pressures shaping the current populations, including an assessment of population level differentiation, population fragmentation, and population genetic structure. In this study we generated the highest quality *de novo* genome assembly (2.65 Gb, with a scaffold N50 of 69.17 Mb) of African buffalo to date, and sequenced a further 195 genomes from populations representing the distribution of all subspecies. Principal component and admixture analyses provided surprisingly little support for the currently described four subspecies, but indicated three main lineages, clustered on Western/Central, Eastern and Southern Africa, respectively. Estimating Effective Migration Surfaces analysis suggested that geographical barriers across the continent have played a significant role in shaping gene flow and the population structure. Estimated effective population sizes (N_e) indicated a substantial drop in N_e occurring in all populations 5-10,000 years ago, coinciding with the rapid increase in human populations on the continent. Finally, signatures of selection were enriched for key genes associated with the immune response, suggesting infectious disease exert a substantial selective pressure in shaping the genetics of African buffalo. The data is suggestive of protozoan parasites (the African buffalo is the primary host for the tick-borne *Theileria parva*, an important pathogen of cattle) perhaps exerting particularly strong selection. These findings have important implications for understanding bovid evolution, buffalo conservation and population management, and provide a route to identifying the pathways involved in bovid tolerance to pathogens.

Poster Session 3 – Monday 20 November

Theme 15. Teaching immunology

214 - African Vaccinology Network (AfVANET): an African network by African scientists

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African population has paid and is always paying heavy toll to infectious diseases throughout history. Despite immense progresses in the prevention, detection and treatment of infections, the emergence of new and re-emergence of old diseases, compounded with the increase of antimicrobial resistance makes many infectious diseases a serious concern for the continent. Recognition of this fact has led to the concept of One Health in which human, environmental and animal health, including for wildlife and domestic species, is addressed in an integrated global approach. However, management and control of infectious diseases to be efficient at the global scale needs a reduction of the gap between developed and developing regions of the world. Young scientists are crucial for Africa's advancement and a necessity for the continent's economic development. Yet, they face persistent barriers to success that cause them to leave their home countries, or abandon a research career (Nature Africa, 2021). Existing data on the challenges facing the African research system highlight major structural contributors: low but fast-growing enrolment rates, under-funding and gross inefficiencies, low research output and brain drain, and insufficient quality assurance. The economic gap between developing and developed countries is bigger than ever, and this has consequences for public health. So, to sustain education and research in the most resource-constrained regions, it is necessary to promote local teaching of the immunology of infectious disease (Nature reviews, 2005). Despite the efforts, unfortunately, immunology remains and under-represented science in terms of number of countries where teaching is available. Most other African Universities offer lessons in basic immunology and have 1-5-week workshops teaching immunology and vaccinology. Many of the individual projects have been impressive successes, but even many of the most successful aren not connected to the full range of expertise needed to get to the point of impact.

Theme 17. Toolkit Workshop

017 - Fc-tagged fusion proteins as tools to define veterinary cytokines and investigate immunological receptor/ligand interactions

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The availability of recombinant cytokines and chemokines, and methods to detect expression of their receptors is important to enable studies of a range of immune cell types and their functions. However, development of monoclonal antibodies to complex immune receptor families is difficult and not widely reported for veterinary species. In addition, significant gaps in reagent availability hamper studies of the role of cytokines in cell differentiation and function. Here, we report on the utilisation of recombinant Fc-tagged purified bovine cytokines, with examples including FLT3L, XCL1, CSF-1 and RANKL. Using a factor-dependent Ba/F3 cell line transfected with individual cognate receptors, we have demonstrated that bovine FLT3L-Fc can specifically bind to cellular receptor FLT3, a molecule which plays a crucial role in haematopoiesis and myeloid cell subset definition and bovine XCL1-Fc binds specifically to the G coupled receptor XCR1, a marker of cross presenting dendritic cells. Furthermore, we have shown that both bovine CSF-1-Fc and bovine FLT3L-Fc are capable of driving proliferation of Ba/F3 cells independently of IL-3, confirming they are biologically active. Using confocal microscopy we have been able to demonstrate that bovine RANKL-Fc can induce differentiation of bovine and sheep monocyte/macrophage into osteoclasts confirming biological activity. The development

of a range of biologically active cytokines and chemokines that can also be utilised to identify receptor expression will facilitate additional studies of the phenotype and function of bovine cytokines. This could be widely applicable as a strategy for other livestock, and wildlife species.

Poster Session 2 – Sunday 19 November

101 - Generation and characterization of swine immune reagents for monitoring pig immune status and for biomedical research

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Developing new swine immune reagents is a priority to strengthen the swine immune research. The USDA-NIFA Swine Immune Toolkit Initiative has been involved in generating priority immune reagents for understanding swine immunity, facilitating biomedical research, and pipelining resultant tools to marketing. With the help of commercial partners, we expressed each target immune molecule as a soluble protein using yeast expression system and then produced panels of monoclonal antibodies (mAbs) against each target. Most recently we successfully generated panels of mAbs reactive to porcine IL-6, IL-13, IL-28B, CXCL10, and BAFF. We determined mAb reactivity to orthologous proteins for most panels of mAbs. A sensitive sandwich ELISA is now available for IL-13 and CXCL10; other targets are being screened for best mAb pairs. Reactivity tests for intracellular staining of porcine immune cells using flow cytometry assay for labeled α-CXCL10 was successful and is underway for α-IL-6 and α-IL-13 mAbs using different cell stimulation conditions. Immunohistochemistry analyses for binding of two of the α-CXCL10 mAb on formalin fixed pig lymph nodes and spleen tissues was confirmed successfully. Further, analysis of a large panel of commercial α-human cluster of differentiation (CD) antigen mAbs for cross-reactivity with porcine cells is ongoing. For each target, our goal is to provide the veterinary community with new commercial reagents and standardized assay techniques for their research efforts. Finally, efforts are proceeding to characterize the swine leukocyte antigen (SLA) tetramers for antigen presentation studies. Tools and reagents generated by this project will undoubtedly advance our understanding of swine immune responses to disease and vaccine and use for biomedical research efforts.

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Poster Session 2 – Sunday 19 November

136 - CD25, CD40L and CD69 as activation induced markers (AIMs) in porcine T cells

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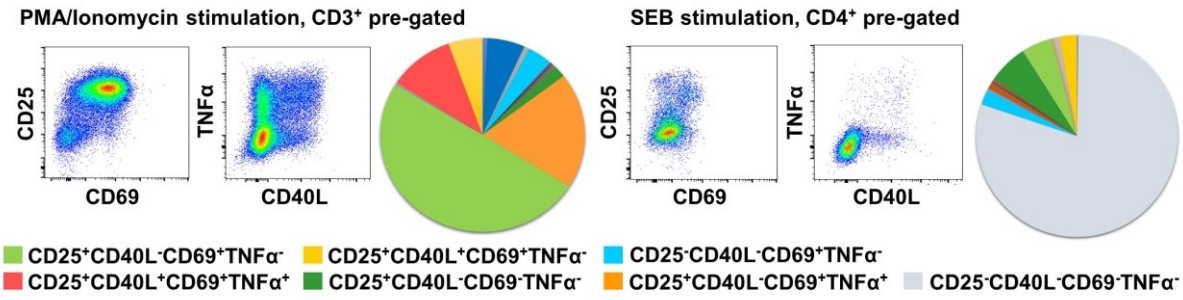
Activation induced markers (AIMs) have become a routine target in the study of human T cell responses, for example during COVID-19 vaccination studies. However, in pigs the analysis of AIMs is still not very common. Based on available antibodies, we designed a multi-colour flow cytometry panel comprising pig-specific or cross-reactive antibodies against CD25, CD69 and CD40L (CD154) and combined it with lineage/ surface markers against CD3, CD4 and CD8alpha. In addition, we included an antibody against TNFα, to study the correlation of AIM expression with the production of this abundant T cell cytokine. The panel was initially optimised with PMA/Ionomycin stimulated PBMCs. Following this stimulation, a substantial amount of responding CD3+ T cells showed a co-expression of CD25 and CD69. The second-most prominent phenotype were CD25+CD69+TNFα+ cells. Cells expressing all three AIMs and TNFα were also prominent but varied more

strongly between pigs. We also tested Staphylococcus enterotoxin B for stimulation. When CD4+ T cells were gated, the most prominent phenotype were cells that had upregulated CD25 only, followed by cells that co-expressed CD25 and CD69. Other prominent phenotypes were CD25+CD40L+CD69+ and CD40L-single positive, but here a stronger animal-to-animal variation was observed. Currently, we are in the process of testing the panel with T cells from influenza A virus and African swine fever virus primed pigs. In summary, this combination of AIMs will allow the identification of primed T cells beyond the commonly used cytokine panels, improving capabilities to identify the full breadth of antigen-specific T cells in pigs.



Poster Session 2 – Sunday 19 November

148 - Comparing flow cytometry and bulk transcriptomics with single cell gene expression to characterize cattle B cell subsets

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Flow cytometry is a powerful tool for in-depth phenotypic and functional analysis of B cells but is dependent on available species-specific reagents. We have developed an 8-color panel using characterized antibodies against cattle cell surface molecules (immunoglobulin light chain (S-Ig[L]), CD20, CD21, CD40, CD71, and CD138) to discriminate 24 unique cell subsets within the B-cell population and identify five putative functionally distinct B-cell subsets critical to infection and vaccination responses: (1) naïve B cells, (2) regulatory B cells, (3) memory B cells, (4) plasmablasts, and (5) plasma cells. To obtain a more thorough understanding of the gene transcription and protein expression dynamics and their correlation within the different compartments of B-cell subsets in cattle, we employed a combined approach using flow cytometry and RNA-seq analysis. Concurrently, we used the 10X Genomics platform for single-cell gene expression analysis to validate the key markers that distinguish and categorize B cell clusters. By employing these approaches, we seek to verify the markers specific to the B-cell subsets of interest, acquire a comprehensive understanding of their unique characteristics, and facilitate the monitoring of B-cell responses to infection or vaccination in cattle.

Poster Session 2 – Sunday 19 November

- - END POSTER PRESENTATIONS - -

Workshops

Pushing boundaries in veterinary immunology - A new era of NGS, with single cell sequencing and spatial transcriptomics

Redefining NGS, one cell at a time. Join us for a half day workshop at IVIS and learn how single cell sequencing is changing the future and leading to fundamental discoveries across multiple areas of biology, including oncology and immunology.

Every day, immunologists deepen our understanding of the immune system's intricate network of responses and interactions. Learn how single cell and spatial multiomics are empowering exciting new research into autoimmunity, immuno-oncology, vaccine development, and beyond.

Explore methods that let you uncover molecular insights, dissect cell-type differences, detect novel cell subtypes and biomarkers, define gene regulatory interactions, and decipher spatiotemporal gene expression patterns.

This workshop will be hosted by renowned researchers in veterinary immunology, Prof Christine Maritz-Oliver and Prof Crystal Loving, as well as supported by Diagnostech and 10x Genomics experts.

We will cover topics like:

- What is Single Cell Sequencing and why are we heading in this direction?
- What is the technology, and what are the limitations?
- How can you successfully design a project using these technologies, and get workable results to publish?
- Bioinformatics, computational difficulties and what to master early on
- Discussion with the experts

Friday 17 November | 08:30

Novel approaches to the development of anti-bacterial veterinary vaccines

This workshop will review vaccine platform technologies that can be deployed for development of veterinary bacterial vaccines through short presentations by expert speakers followed by breakout discussions in small participant groups. The breakouts are designed to identify gaps in bacterial vaccine development and discuss how these gaps can be addressed using novel vaccine platform technologies. The focus will be on development of fit-for-purpose vaccines with a target product profile that meets the needs of the relevant end-users, including vaccines that will be deployed in LMICs. The outcomes of the individual group discussions will be presented back to the whole workshop delegation in a final Q&A session. The workshop findings will be summarised in a report that can be used to inform on future directions for veterinary bacterial vaccinology research.

This workshop is supported by UKRI BBSRC, STAR-IDAZ, The Methane Hub, Defra, IUIS VIC and the Vaccinology Networks IVVN, VALIDATE and BactiVac.

Draft Programme

12.30 -12.40: Introduction and Aims (Chairs: Jayne Hope and Gary Entrican)
12.40 - 13.50: Short Presentations on Vaccine Development and Vaccine Platform Technologies
12.40: Paul Wood (Monash University, Australia): Importance of designing the vaccine TPP for translational research
12.50: Adam Cunningham (BactiVac, UK): Outer membrane vesicles (OMVs) for bacterial vaccinology

13.00: Brendan Wren (LSHTM, UK): Bacterial glycoconjugate vaccines (delivered by the panel)
13.10: Michael Jarvis (The Vaccine Group, UK): Viral vectors for veterinary vaccinology
13.20: Helba Bredell (Afrigen, SA): mRNA platforms for veterinary vaccinology
13.30: Gary Entrican (University of Edinburgh, UK): STAR-IDAZ vaccine roadmaps
13.40: Johannes Charlier (Kreavet, Belgium): DISCONTOOLS bacterial vaccines gaps
13.50 – 14.10: Initial Q&A, format of Breakout Groups, Assembly and Begin Discussions (All, Rapporteurs and Questions TBC*)
14.10 – 14.30: Coffee/Networking/Opportunity for Initial Informal Feedback (All)
14.30 – 15.10: Breakout Discussions (50min)
15.10 – 15.40: Rapporteur Summaries (TBC, 5 min maximum/one slide each, estimated timings**)
15.40 – 16.10: Panel Discussion with Speakers and Chairs
16.10 – 16.30: Capture the Agreed Workshop Outcomes, Final Comments and Close (Chairs)

Expected Outcomes:

- Identify gaps in veterinary vaccinology for bacterial diseases
- Comments that inform on functionality of STAR-IDAZ roadmaps
- Highlight opportunities to address these gaps using novel vaccine platform technologies with a One Health approach
- Maintain awareness of the TPP at all stages of research to develop vaccines that meet end-user needs (especially for LMICs)
- Prepare an informed, positional report that informs funders and networks on areas for funding future research

Friday 17 November | 12:30

Immunological Toolkit Session

The Immunological Toolkit session will provide an overview of ongoing work in reagent development and novel technologies and techniques. These will be followed by a discussion session to discuss future priorities and gap analyses in reagent and technology development.

Draft Programme
08h30: Hamid, Benjamin-Layla - (071) CD38 expression on porcine αβ-T-cell subsets and its role in T-cell activation
08h40: Dry, Inga - (037) The Immunological Toolbox: Advancing veterinary immunology research through the generation of novel reagents
08h50: Di Placido, Marie - (147) A customizable suite of methods to sequence and annotate cattle antibodies
09h00: Entrican, Gary - Recombinant antibodies driving immunological research
09h10: Hammond, John - The future of the Toolbox
09h20: Round Table facilitated discussions with pre-shared questions
09h50: Feedback from discussions via rapporteurs

Sunday 19 November | 08:30

VIC MHC

Draft Programme
10h30: Hammer, Sabine E. - (020) Comparative analysis of swine leukocyte antigen (SLA) gene diversity in Göttingen Minipigs
10h48: Robert, Jacques - (067) Role of nonpolymorphic MHC-I and innate-like T cells in resistance and tolerigenic neonatal immunity to mycobacteria
11h06: Rogel-Gaillard, Claire - (129) A revised view of putative functional histocompatibility genes in the SLA complex

11h24: Schwartz, John C - (126) Haplotypic and allelic diversity of non-classical MHC class I in ruminants
11h42: Hammond, John - An update on species and tools on IPD-MHC

Sunday 19 November | 10:30

Supporting early career researchers in veterinary immunology and vaccinology research

This workshop, hosted by the International Veterinary Vaccinology Network (IVVN), will focus on ways in which we can support early career researchers working in veterinary immunology and vaccinology research. The workshop will be facilitated by a group of IVVN ECR members who will present a white paper which outlines the challenges faced by early career researchers and potential solutions to mitigate these challenges. In addition, there will be presentations from representatives of Afrique One Aspire, Bill and Melinda Gates Foundation (BMGF), The African Academy of Sciences (AAS) and The African Research Universities Alliance (ARUA). All early career researchers, and those who are keen to support the next generation of veterinary immunologists and vaccinologists, are very welcome to join.

Tuesday 21 November | 11:00

Thank you for joining us for the 13TH International Veterinary Immunology Symposium

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