IVVN Symposium

VACCINES FOR RUMINANTS

26 April 2021
9am to 12.30pm BST

Programme and abstracts

Contents

Programme

Session chairs: **Professor Bryan Charleston** (The Pirbright Institute, UK)
and **Professor Yanmin Li** (Southwest Minzu University, China)

09.00 – 09.05	**Welcome and introduction** Dr Timothy Connelley (IVVN Director, The Roslin Institute, UK)
09.05 – 09.35	**High expression of an echinococcosis vaccine antigen EG95 in yeasts and its application** Professor Wanzhong Jia (Lanzhou Veterinary Research Institute, China)
09.35 – 10.05	**FMD vaccination in South-East Asia** Dr Yu Qiu (OIE South-East Asia and China Foot-and-Mouth Disease Campaign, Thailand)
10.05 – 10.35	**BCG and bovine tuberculosis: why perfect is the enemy of the simply good** Professor Vivek Kapur (Pennsylvania State University, United States)
10.35 – 11.05	**Break and poster session** in breakout groups
11.05 – 11.25	**Development and evaluation of a multiepitopic protein as vaccine candidate against bovine babesiosis caused by *Babesia bigemina*** Professor Juan Mosqueda (Natural Sciences College Autonomous University of Querétaro Mexico)
11.25 – 11.45	**Q-fever: the use of a pregnant ewe challenge model to assess the efficacy of phase I and phase II Coxiella burnetii bacterin vaccines in sheep** Dr Tom McNeilly (Moredun Research Institute, UK)
11.45– 12.05	IVVN pump-priming grant: **Isolation and purification of Nairobi sheep disease virus for development of a thermostable vaccine** Dr Caroline Wasonga (University of Nairobi, Kenya)
12.05 – 12.25	IVVN pump-priming grant: **Construction of foot-and-mouth disease (FMD) virus-specific phage-display libraries and epitope identification for improved FMD vaccines generation** Dr Pamela Opperman (ARC-Onderstepoort Veterinary Research, South Africa)
12.25 – 12.30	**Close**

Sponsors

zoetis

THE BLOOMSBURY SET®
Science | Economics | Technology

Phibro™

Speaker abstracts

High expression of an echinococcosis vaccine antigen EG95 in yeasts and its application

Professor Wanzhong Jia

Lanzhou Veterinary Research Institute, China

Cystic echinococcosis (CE), one of the parasitic zoonoses, which is caused by the larval stage of *Echinococcus granulosus*, may cause not only morbidity, disability and death in humans, but also substantial economic losses in animal husbandry. Breakthroughs have been made towards the development of practical and recombinant vaccines in sheep and cattle to interrupt the transmission of disease from dogs to animals and humans. For example, experimental studies and trials or field trials with the EG95 vaccine, performed in sheep and cattle against experimental challenge infection and natural infection with *E. granulosus* respectively in New Zealand, Australia, Argentina, China and other countries, demonstrated that EG95 vaccination conferred a high protection against *E. granulosus* infections or echinococcosis. Up to date, EG95 antigen has been expressed in *Escherichia coli* as glutathione S-transferase (GST) or His-tag fusion proteins. However, another taeniid protective antigen, designed TSOL18, was highly produced in *Pastoris pichia* in a secreted and glycosylated form described by CAI XP et al (2008), which closely resembles the native TSOL18 antigen in *Taenia solium* oncospheres.

In our work, production of EG95 antigen using *P. pichia* and its antigenicity were explored. The results were as following: (1) Genetically modified *P. pichia* strains were constructed and screened with high level of secreted expression EG95 antigen. Under high density fermentation (a 5L fermenter) conditions, total protein content per litre of culture supernatant reached over 4 grams (BCA method) with a purity of approximate 90% EG95 antigen. (2) N-glycosylation of recombinant EG95 antigen could be changed or controlled through carbon sources etc. (3) EG95 antigen from yeasts performed good immunogenicity, which could stimulate higher production of antibodies than GST-EG95 antigen. (4) Indirect ELISA kits based on purified *P. pichia* origin of EG95 antigen had much better performance in detecting anti-EG95 antibodies in vaccinated animals than those based on purified E. coli origin of EG95-His fusion antigen.

In conclusion, a high level of EG95 expression based-on yeast system has been successfully developed. Such antigen expression technology based on yeast cells has potential to produce taeniid protective glycoproteins such as TSOL18 and EG95. The EG95 antigen is being used for the development of novel engineered vaccines against ovine and bovine echinococcosis and ELISA detection kits for the vaccinated animals. Also, purified EG95 antigen without tags has potential to develop vaccines for human usage.

FMD vaccination in South-East Asia

Dr Yu Qiu

OIE Sub-Regional Representation for South-East Asia, Thailand

FMD control in South-East Asia is through a regional approach under the OIE SEACFMD Campaign (https://rr-asia.oie.int/en/projects/fmd/seacfmd-campaign/). Vaccination is an important tool of FMD control in addition to biosecurity and movement control. In South-East Asia, FMD vaccines used are of both imported and locally produced sources. Resource deficit countries including Lao PDR, Cambodia and Myanmar have limited access to high-quality vaccines and mainly rely on vaccines supplied by international donors. Although Myanmar has a small FMD vaccine production facility using the local serotype O virus, the production capacity could hardly cover 1% of the total cattle population and thus exert a limited impact on the overall disease control. Thailand produces its own trivalent FMD vaccines for mass vaccination in large ruminants twice (in beef cattle) or three times (in dairy cattle) a year, and the vaccine strains are under continuous monitoring by the OIE FMD reference laboratory in Pakchong to ensure antigenic match with field circulating viruses. Locally produced bivalent vaccines are also supplied to the pig sector, while depending on the capacity of the local plant, some of the vaccines used are supplied from oversea manufacturers. In peninsular Malaysia, imported trivalent vaccines are used in potential high-risk areas to increase population immunity level in cattle herds, and also used to conduct emergency vaccination during an outbreak. Vietnam conducts mass vaccination in high-risk areas along international borders using bivalent vaccine, and uses monovalent type O vaccines for endemic disease control. Started in 2007, Vietnam has made a contract with a European commercial vaccine manufacturer to supply antigen concentrate, and the reconstitution and bottling are managed locally in Vietnam. Since 2018, Vietnam has started to produce monovalent type O FMD vaccines using local strains to meet the increasing domestic market demands.

While vaccine strains selection in vaccine producing countries is performed by the national FMD laboratory of each country, the strains requested from international suppliers are usually based on the recommendations of the OIE/FAO World Reference Laboratory for FMD (Table 1). For many years FMD vaccine quality control has not been progressed significantly in South-East Asia due to technical and financial constraints. Imported vaccines are assessed based on data supplied by manufactures without local validation. In Thailand, the FMD production plant conducts its own vaccine quality control on some batches without external validation. In Myanmar, quality control is restricted to tissue culture inoculation and sterility tests without animal challenge studies. The need to address the gaps in vaccine quality control has been underlined by SEACFMD Member Countries and a regional approach will be developed under the new SEACFMD Roadmap 2021-2025.

The OIE and FAO have developed the FMD vaccination and post-vaccination monitoring (PVM) guidelines and encourage countries to use the PVM findings to further improve vaccination strategies. Given the high-level of virus prevalence in infected countries in South-East Asia, the PVM study has mainly been limited to the assessment of magnitude and persistence of vaccine-induced immunity. Initial findings from limited studies show a higher-level and longer-duration of antibody response elicited by imported international vaccines compared to locally produced vaccines.

Table 1 FMD vaccine strains used in South-East Asia

Country	Vaccine strains	Source
Thailand	O/Udonthani/87 A/Sakonnakorn/97 Asia-1/Petchburi/1985	Locally produced
Malaysia	O1/Manisa O/3039 A/Malaysia 97 Asia 1/Shamir	Imported
Vietnam	O1/Manisa O/3039 A22/Iraq	Imported
Myanmar	O/3039 A/Malaysia/97 A22/Iraq Asia 1/Shamir	OIE Vaccine Bank
Lao PDR	O/3039 A/Malaysia/97 A22/Iraq	OIE Vaccine Bank

BCG and bovine tuberculosis: Why perfect is the enemy of the simply good

Professor Vivek Kapur

Pennsylvania State University, United States

Bovine tuberculosis (bTB)is a wicked problem. The test-and-remove approach that successfully enabled control of bTB in many parts of the world is economically and socially unimplementable in many low-and-middle countries where the disease is endemic. So, what might one do? BCG has long been recognized as an imperfect vaccine - and since vaccinated animals cross-reacted with the prescribed intradermal skin test, it's use in control programs was precluded. This presentation will highlight the approach we are taking to cutting this Gordian knot, and why perhaps, with respect to BCG use in control of bTB – perfect may indeed be the enemy of the simply good.

Development and evaluation of a multiepitopic protein as vaccine candidate against bovine babesiosis caused by *Babesia bigemina*

Juan Mosqueda[1,2], Diego Josimar Hernández-Silva[1], Cristian Figueroa-Banegas[1], Bertha I Carvajal-Gamez[1] and Gabriela Aguilar-Tipacamú[2]

***Affiliations: 1** Immunology and Vaccines Laboratory, Natural Sciences College, Autonomous University of Queretaro, Queretaro, Mexico; **2** Animal Health and Environmental Microbiology, Natural Sciences College, Autonomous University of Queretaro, Queretaro, Mexico*

Bovine babesiosis is a disease that impacts livestock industry worldwide. In the Americas, it is caused by *Babesia bigemina* and *Babesia bovis*. There is no recombinant vaccine for the prevention of bovine babesiosis. In this study, a chimeric gene was constructed using the coding sequence of 21 peptides from AMA1, MIC1, RAP-1α1, RON2, SBP4 and HAP2 proteins of *B. bigemina*, each one of them is conserved among strains and contain neutralizing, B-cell epitopes. Expression of the multiepitopic protein was induced in a bacterial system and it was recognized by anti-serum against each of the 21 peptides.

The recombinant protein (Chp1.9, patent registry in process) was evaluated as a vaccine against *Babesia bigemina*. Five cattle were immunized with Chp1.9 three times every 21 days using 100µg of protein in adjuvant. Another five cattle were only immunized with adjuvant. The efficacy of the vaccine was evaluated by challenging all animals with 1×108 parasitized erythrocytes (PE) of a *Babesia bigemina* field isolate and measuring the rectal temperature (TR), agglomerated cell volume (VCA) and percentage of PE (PPE) for 10 days post-infection. The PPE of the vaccinated group was significantly lower ($p < 0.5$) compared to the control group and the number of days where parasitemia was observed were fewer. Most of the animals in the control group had a fever for three days or more and a reduction in VCA of 40% or more, while no animals in the vaccinated group reached any of these conditions. On day 11 post infection the animals of the control group were treated while no animals of the vaccinated group required treatment. Immunization with Chp1.9 conferred protection to the controlled challenge against *B. bigemina* in susceptible cattle and can be considered a vaccine candidate against this disease.

Q-fever: the use of a pregnant ewe challenge model to assess the efficacy of phase I and phase II Coxiella burnetii bacterin vaccines in sheep

Sarah E Williams-Macdonald[1], Mairi Mitchell[1], Bill Golde[1], David Longbottom[1], Alasdair J Nisbet[1], Søren Buus[2], Hendrik-Jan Roest[3], Ad P Koets[3], Annemieke Dinkla[3] and Tom N McNeily[1]

***Affiliations:* 1** *Disease Control, Moredun Research Institute, Edinburgh, UK;* **2** *Department of Immunology and Microbiology, University of Copenhagen, Denmark;* **3** *Central Veterinary Institute, Wageningen University & Research, Wageningen, Netherlands*

Q-fever is a highly contagious zoonotic disease caused by the bacterium, *Coxiella burnetii*. Domestic ruminants, notably sheep and goats, are the primary reservoir of human infection, as the bacterium is excreted at high loads during parturition. Human infection occurs via inhalation of as few as 1-10 aerosolized bacteria and can result in both acute and chronic forms of disease. In livestock, particularly sheep and goats, infection can cause abortion, stillbirth and delivery of weak offspring, resulting in substantial economic losses.

Vaccines based on the virulent phase I antigenic variant of *C. burnetii* are currently the most effective way to control *C. burnetii* in humans and livestock; however, these vaccines are difficult to manufacture and are highly reactogenic. Vaccines based on the avirulent phase II variant of *C. burnetii* fail to induce protection in goats and mice, but their effectiveness in sheep is unknown.

Prior to mating ewes (n = 6 per group) were vaccinated using a commercial phase I vaccine (Coxevac®), an experimental phase II vaccine or were non-vaccinated. All ewes were challenged with 106 Infectious Mouse Doses of the nine-mile strain of phase I *C. burnetii* at day 95 of gestation. The presence of *C. burnetii* in milk, faeces and vaginal swabs post-lambing was determined by PCR.

Levels of *C. burnetii* were significantly lower in faeces ($p = 0.030$), milk ($p = 0.0008$) and vaginal-swab samples ($p = 0.002$) of vaccinated ewes compared to non-vaccinated controls. A significantly higher proportion of abnormal pregnancies were observed in non-vaccinated ewes compared to phase I and II vaccinated ewes ($p = 0.01$). There were no differences between phase I and phase II vaccinated animals for any of the above parameters.

Protection elicited by the non-virulent phase II vaccine could potentially offer a safer and more easily manufactured alternative to virulent phase I vaccines for use in sheep.

IVVN PUMP-PRIMING GRANT

Isolation and purification of Nairobi sheep disease virus for development of a thermostable vaccine

Dr Caroline Wasonga[1], Dr Joel Lutomiah[2], Dr Jennifer Duncan[3], Dr Michael Muthamia Kiraithe[4], Dr Allan Ole Kwallah[2] and Edna M Ondari[2]

***Affiliations:** **1** University of Nairobi, Kenya; **2** Kenya Medical Research Institute, Kenya; **3** University of Liverpool, UK; **4** Kenya Agricultural and Livestock Research Organization, Kenya*

Nairobi sheep disease virus (NSDV) causes severe illness in sheep and goats, with fatalities reaching up to 90% in affected animals. This disease is widespread in Eastern Africa and in Indian subcontinent where it is known as Ganjam virus. It causes considerable losses, especially to small scale farmers. Sheep and goats are also an important source of proteins and provide income through sales. The control of NSDV will thus make a positive contribution to the rural poor. This project aims to contribute towards control and eradication of NSDV, by developing a vaccine that is effective against NSDV. Field studies will be done to obtain tick samples, NSDV isolated from the ticks, and grow in the laboratory. The purified NSDV isolates will be inactivated using formalin to generate an inactivated vaccine candidate and this vaccine will then be tested in sheep to confirm that the vaccine is indeed protective against NSDV.

IVVN PUMP-PRIMING GRANT

Construction of foot-and-mouth disease (FMD) virus-specific phage-display libraries and epitope identification for improved FMD vaccines generation

Dr Pamela Opperman[1], Dr Melanie Chitray[1], Dr Francois Maree[1], Dr Alejandra Capozzo[2], Dr Mariano Perez-Filgueira[2], Dr Anna Ludi[3], Dr Richard Reeve[4]

***Affiliations:** **1** ARC-Onderstepoort Veterinary Research, South Africa; **2** National Agricultural Technology Institute, Argentina; **3** The Pirbright Institute, United Kingdom; **4** University of Glasgow, United Kingdom*

Foot-and-mouth disease (FMD) ranks as one of the most economically important infectious diseases of cloven-hoofed animals and is endemic in large regions of Africa, Asia and the Northern part of South America. The majority of FMD virus (FMDV)-neutralizing antibodies are directed against epitopes located on the surface-exposed capsid proteins. However, the location of these antigenic sites and their antigenic features may vary between the different strains. Antigenic variation results from changes to the viral capsid because of the high mutation rate of the virus such that each FMDV isolate is antigenically unique in its fine epitopic composition. This process leads to the generation of new variants circulating in the field, which may be different from those included in the vaccines. Therefore, vaccine efficacy and effectiveness of vaccination programs may be dramatically affected. Knowledge of the amino acid residues that comprise the antigenic determinants of FMD viruses is essential for the rational design of vaccine virus seeds that antigenically match circulating emerging or re-emerging strains, as well as induce a broad immunological response. Monoclonal antibodies, traditionally used for mapping of viral epitopes, have several limitations such as low throughput and high cost, which renders this technology unappealing. This project aims to identify critical antigenic determinants within the FMDV capsid from strains that circulate in different parts of the

world, using newly developed assays. It will join the complementary expertise of four FMDV research institutes: ARC-OVR (South Africa), the Pirbright Institute (UK), INTA (Argentina) and the University of Glasgow (UK). We aim to use a library of recombinant “mini”-antibodies generated from FMDV-immune buffalos and cattle by making use of a phage-display library technology, which has been extensively and successfully used by ARC-OVR. This joint-project will provide important information by identifying critical FMDV epitopes, unique or shared among different strains, which can be implemented to produce improved vaccines.

Poster abstracts

1 Investigation of the *Theileria parva* surface glycan repertoire and binding C-type lectin receptors

J. Kolakowski[1], B. Lepenies[2], D. Lindenwald[2], H. Harris[1], D. Ngugi[1], B. Shiels[3], T. Connelley[4], D. Werling[1]

***Affiliations:** **1** Royal Veterinary College, Hatfield, United Kingdom, **2** Immunology Unit and Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Hannover, Germany, **3** Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, Scotland, **4** Roslin Institute, University of Edinburgh, Scotland*

East Coast Fever (ECF), caused by the apicomplexan parasite Theileria parva, kills more than one million cattle every year and remains the most important tick-borne disease of cattle in sub-Saharan Africa. Disease prevention relies on the costly, complex and laborious “Infection and Treatment Method” (ITM) which results in a parasite strain specific, MHC class I restricted, cytotoxic T cell response. Previous attempts to develop subunit vaccines for a broader immune protection have only paid comparatively little attention to molecules other than proteins. Carbohydrate-based vaccines have been used very successfully in human medicine and are more and more developed for farm animals. A recent re-annotation of the Theileria parva genome suggests glycosylation in the parasite yet nothing is known about the composition or existence of its surface glycan repertoire. In this study, we confirm the presence of carbohydrate-based targets on the surface of two parasitic life cycle stages in the cattle host, sporozoites and schizonts. Such targets were detected using recombinant C-type lectin receptor fusion proteins in a flow cytometric assay. Interestingly, the binding pattern of the recombinant proteins suggested a surface glycan repertoire specific for each of the two life cycle stages. Characterising such surface glycans and investigating their role in bovine host cell infection and transformation will allow for the identification of potential vaccine candidates.

2 An invariant *Trypanosoma vivax* vaccine antigen eliciting protective immunity

Delphine Autheman[1], Cecile Crosnier[1], Simon Clare[2], David A. Goulding[3], and Gavin J. Wright[1,4]

***Affiliations:** **1** Cell Surface Signalling Laboratory, **2** Pathogen Support Team, **3** Electron and Advanced Light Microscopy, Wellcome Sanger Institute, Hinxton, Cambridge; **4** Department of Biology, York Biomedical Research Institute, York*

Animal African Trypanosomiasis (AAT) has a significant impact on animal agriculture in Sub-Saharan Africa by threatening the livelihood of farmers and food security in endemic countries, and is mainly caused by two species of African trypanosomes, Trypanosoma congolense and T. vivax. While vaccination would be an ideal solution to manage AAT, effective vaccines against African trypanosomes were considered unachievable, primarily due to the sophisticated system of antigenic variation employed by these pathogens to elude the host immune response.

We have used a reverse vaccinology approach to identify antigenically invariant proteins on the surface of T. vivax parasites to be used as vaccine targets. We assembled a library of forty proteins predicted to be associated with the surface of the parasite bloodstream-forms by expressing the entire extracellular regions as secreted recombinant proteins in mammalian cells to increase the chance that vaccine-elicited antibodies are raised against native parasite epitopes. We systematically vaccinated mice with purified proteins and challenged them with T. vivax parasites to evaluate the proteins’ efficacy as vaccine

candidates. We identified a non-variant antigen (V23) that can elicit sterile protection and demonstrated its localisation to the parasite flagellum-membrane. Naïve mice were also protected from parasite-infection by adoptive transfers of either V23 immune serum or recombinant monoclonal antibodies directed against V23. By individually mutating antibody effector binding sites on protective recombinant antibodies, complement recruitment was found to be a major protective mechanism.
Our results identify this antigen as a leading vaccine candidate against AAT and show that vaccination against trypanosome parasites is achievable.

3 Applying NetBoLAIIpan to the rational screening of vaccine candidates in complex parasites

Andressa Fisch[1], Birkir Reynisson[2], Morten Nielsen[2,3], Nicola Ternette[4], Beatriz Rossetti Ferreira[1] and Tim Connelley[5]

Affiliations: ***1*** *Ribeirão Preto College of Nursing, University of São Paulo, Av Bandeirantes 3900, Ribeirão Preto, Brazil;* ***2*** *Department of Health Technology, Technical University of Denmark, DK-2800 Lyngby, Denmark;* ***3*** *Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, 23 CP1650 San Martín, Argentina;* ***4*** *The Jenner Institute, Nuffield Department of Medicine, Oxford, OX37BN, UK;* ***5*** *The Roslin Institute, Edinburgh, Midlothian EH25 9RG, UK*

R. microplus is a major pathogen of livestock. Vaccine development for ticks often aim to use salivary proteins as antigens, however, ticks are complex parasites that express thousands of salivary proteins, and rationally selecting candidate antigens from a large dataset is a major obstacle. To demonstrate how the newly developed NetBoLAIIpan algorithm could contribute to the rational preliminary selection of candidate antigens from complex pathogens, we have selected the ten most transcribed salivary proteins (PRJNA329522) each from the lipocalins (294 members), Kunitz domain-protease inhibitor (KD-PI) (201 members) and cement-family proteins (49 members) and analysed them to identify peptides predicted to be presented by seven highly prevalent BoLA-DR molecules (BoLA-DRB3 from MS, PRB<1%). For the lipocalins, two proteins contained predicted ligands for 6 BoLA-DR; in the cement family, the maximum number of BoLA-DR molecules covered by any single protein was only 5, and in the KD-PI family, no protein had predicted binders for more than 3 BoLA-DR. On average, proteins from the lipocalin family contained predicted binders for ~4 BoLA-DR, while proteins of cement and KD-PI families contained predicted binders for only ~1 and ~2 BoLA-DR, respectively. Importantly, no single protein was found to have predicted strong binders for all BoLA-DR molecules. To ensure the inclusion of peptides presented by all BoLA-DR molecules evaluated, the combination of peptides from at least two lipocalins or two cement proteins is necessary. However, in KD-PI family, no protein showed predicted binders for two BoLA-DR molecules. In summary, we demonstrated how NetBoLAIIpan can facilitate the selection of candidate antigens by providing predictions of which antigen combinations should facilitate stimulation of CD4 T cells in animals expressing a range of BoLA-DR molecules, representing a rapid, cheap and more rational way of selecting candidate antigens from highly complex pathogens for subsequent in vivo assessment.
Financial Support: FAPESP2018/23579-8.

4 Exploring *Rhipicephalus microplus* salivary proteins to design a B and T cell multi-epitope vaccine applying *in vitro* and *in silico* approaches

Alexsander Moraes[1,2], Andressa Fisch[2], Luiz Gustavo N. Almeida[1], Beatriz R. Ferreira[1,2]

Affiliations: ***1*** *Ribeirao Preto School of Medicine, University of Sao Paulo, Ribeirao Preto, Brazil;* ***2*** *Ribeirao Preto College of Nursing, University of Sao Paulo, Ribeirao Preto, Brazil*

Our group has previously produced a protective anti-*Rhipicephalus microplus* vaccine (77% protection), based on nine salivary antigens. We believe that linear B and T-cell epitopes from these targets could be useful to develop an easier-to-produce multi-epitope vaccine. Using serum from six bovines immunized with the nine-antigen-vaccine, we tested them against all possible linear peptides of each antigen (15aa with 14aa overlapping) in a peptide microarray immunoassay. We identified thirteen linear epitopes from seven antigens recognized by at least four animals. Additionally, we searched for CD4 T-cell epitopes in these seven antigens through NetMHCIIpan3.2, using 27 reference HLA alleles, and selected one epitope of each antigen, which was recognized by the largest number of alleles (3 to 20 alleles). The epitopes identified by both approaches were fused, using flexible (GGGGS), rigid (EAAK) or focused in MHCII processing (KK and GPGPG) linkers to design four multi-epitope antigens (constructions). N-terminal 6xHis-tag and thrombin cleavage sites were added to all sequences. The predicted physicochemical parameters of the constructions (ExPASy ProtParam, SOLpro) indicated that they had a molecular weight ranging from 66.5 to 68.6 kDa; theoretical pI from 8.4 to 9.4; estimated half-life in *E. coli* and yeast after expression of >10h and >20h; hydrophilic (-0.31 to -0.47) and soluble (0.79 to 0.99) constitution. All constructions were predicted to be antigenic (0.77 to 0.78, ANTIGENpro) and non-allergenic (AllerTOP). Results suggest that all the constructs have potential to induce humoral responses, and can be tested as an anti-tick multi-epitope based vaccine to control *R. microplus* infestations.

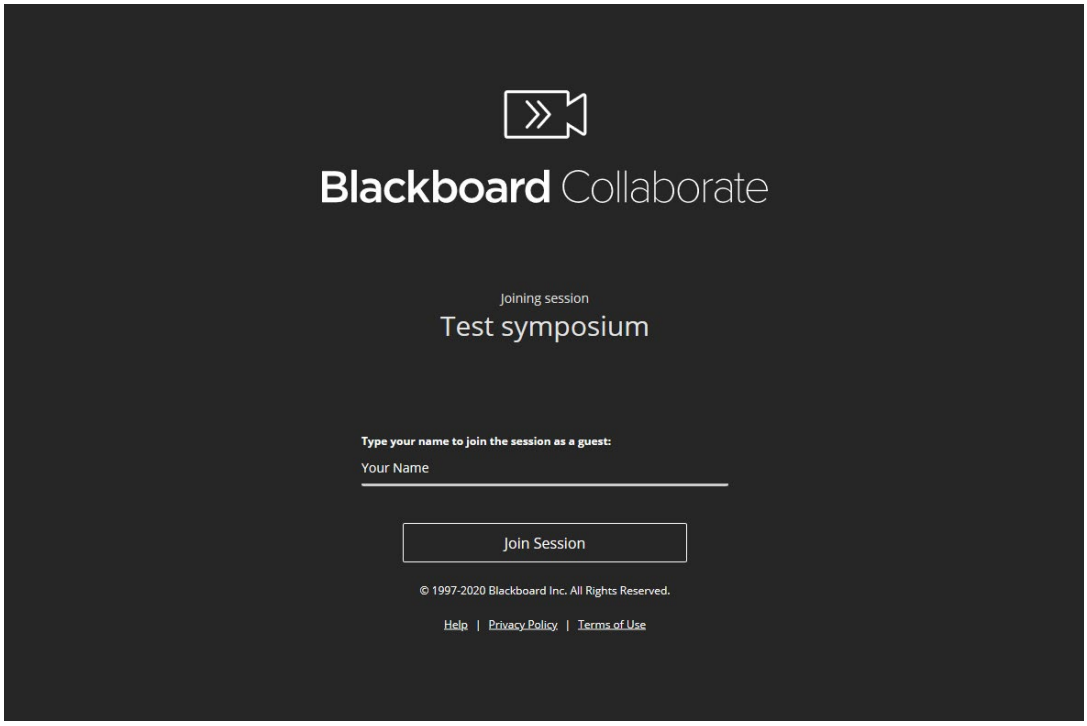
Financial Support: FAPESP (2015/09683-9; 2018/22700-8), CNPq and CAPES.

Blackboard Collaborate: guide for participants

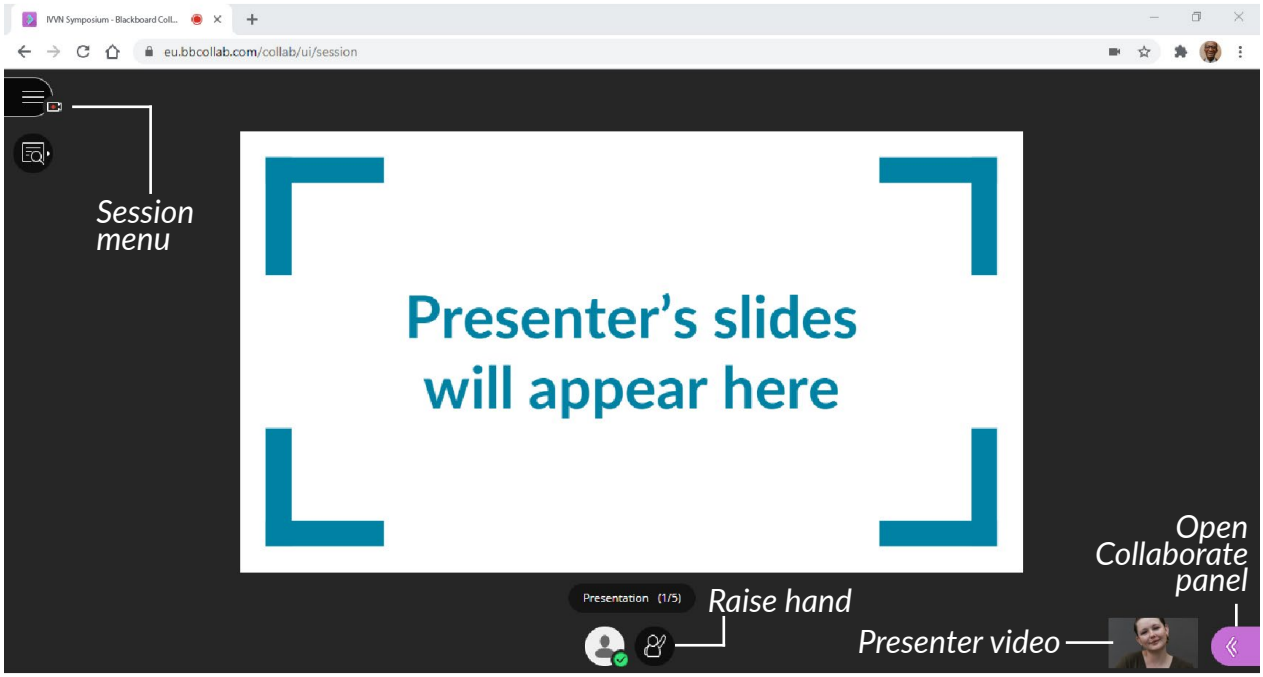
To launch the session, click on the link that was sent to you by email. We recommend that you use Google Chrome or Mozilla Firefox to access the session.

Google Chrome	Mozilla Firefox	Safari	Edge (Chromium)	Edge (older)	Internet Explorer
Recommended	Recommended	Not recommended	Not recommended	Not supported	Not supported

When prompted, enter your name and click 'Join Session'. Entering your full name will allow you to ask questions and participate in breakout groups.



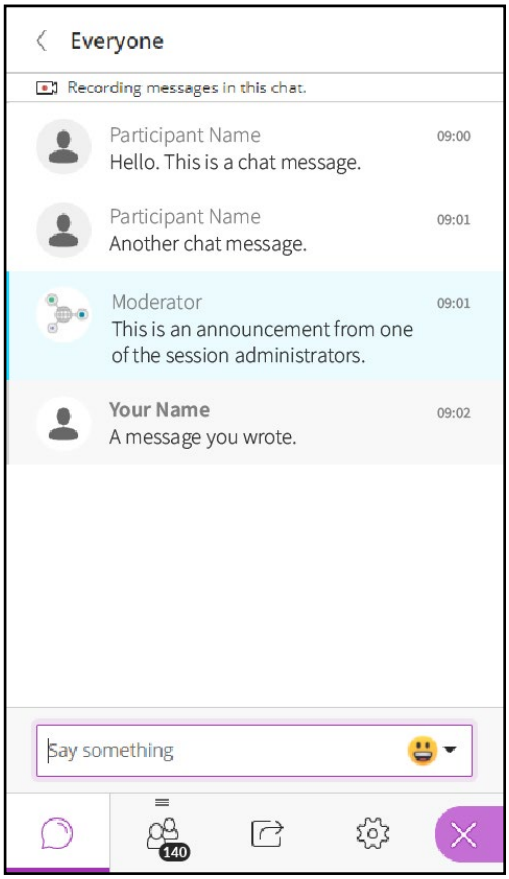
Your browser may ask for permission to use your computer's microphone. This is optional, and your microphone will not be enabled until you choose to unmute yourself during the poster session.



On the main screen, you will see the current presentation being displayed, the presenter's video (if available), a 'raise hand' button to get help from a moderator, and a button on the bottom right to access the Collaborate panel.

Collaborate panel

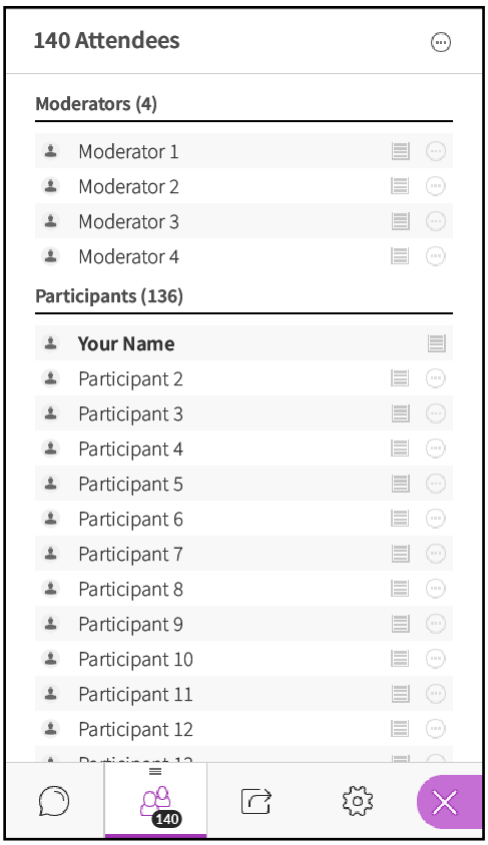
The Collaborate panel is on the right of the screen, and can be toggled using the purple button on the bottom right. Along the bottom of the panel are four options: **Chat**, **Attendees**, **Share Content** and **My Settings**.



Chat

The Chat function is used for text-based messages between attendees. The question-and-answer session following each presentation will take questions from the Chat panel.

Messages from moderators will be highlighted in blue, and messages you send will have a darker background.



Attendees

The Attendees panel shows a list of the session's attendees, grouped according to whether they are Moderators or Participants.

You can use the ⋯ button next to each participant's name to send that participant a private chat message.

My Settings

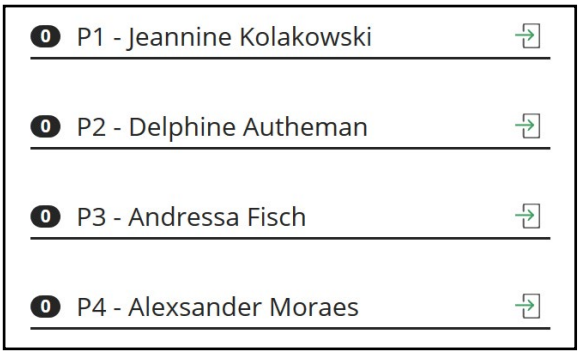
You can use the options in the My Settings panel to adjust your audio and video settings (for the poster session) and to configure notifications.

You may find it useful to silence some or all of these notifications to minimise disruptions during the session.

Poster session

Ahead of the poster and networking session, four 'breakout groups' will open, one for each of the posters that will be presented.

To choose which poster to view, scroll to the bottom of the 'Attendees' panel and click the ⇥ icon next to the name of the speaker.



Entering or leaving a breakout room may be slow, particularly if many participants are attempting to switch groups at the same time (for example at the start of the poster session). You may see 'busy' indicators (shown below) around your microphone and webcam controls while you wait. If this happens, please be patient and wait for the room to load. Please do not refresh your browser.

You can ask the presenters questions using your microphone or using the chat box for each group.

Leaving the symposium

You can leave the session either by closing the window/tab or by clicking the three horizontal lines at the top left of the screen and selecting 'Leave session'.