IVVN Symposium

VACCINES FOR POULTRY

25 February 2021
9am to 12.30pm GMT

Programme and abstracts

co-hosted with

ONE HEALTH POULTRY HUB

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Programme

Session chairs: **Professor Fiona Tomley** (Royal Veterinary College, UK)
and **Professor Hualan Chen** (Harbin Veterinary Research Institute, China)

09.00 – 09.05	**Welcome and introduction** Dr Timothy Connelley (IVVN Director, The Roslin Institute, UK)
09.05 – 09.35	**New approaches for the development of recombinant glycoconjugate vaccines for poultry, pigs and ruminants** Professor Brendan Wren (London School of Hygiene & Tropical Medicine, UK)
09.35 – 10.05	**Successful control of H7N9 influenza in China** Professor Hualan Chen (Harbin Veterinary Research Institute, China)
10.05 – 10.20	**Selectively targeting hemagglutinin antigen to chicken antigen presenting cell receptor induces faster and stronger immunity against avian influenza** Professor Munir Iqbal (The Pirbright Institute UK)
10.20 – 11.00	**Break and poster session** in breakout groups
11.00 – 11.20	**Aerosol Delivery of oligodeoxynucleotides containing CpG motifs (CpG-ODN) at hatch against bacterial septicemia in neonatal broiler chickens under field conditions** Professor Susantha Gomis (University of Saskatchewan, Canada)
11.20 – 11.40	**Identification of avian influenza viruses subtype H9N2 isolated in 2019 from the backyard and commercial farms in Indonesia** Melina Jonas (Medion, Indonesia)
11.40 – 12.00	IVVN pump-priming grant: **Towards edible vaccines for chickens** Dr Kate Sutton (Roslin Institute, UK)
12.00 – 12.20	IVVN pump-priming grant: **Immunogenicity study of matrix 2 ectodomain proteins displayed on nodavirus-like particles as a universal avian influenza virus vaccine for chickens** Dr Mariatulqabtiah Abdul Razak (Universiti Putra Malaysia, Malaysia)
12.20 – 12.30	**Close**

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Speaker abstracts

New approaches for the development of recombinant glycoconjugate vaccines for poultry, pigs and ruminants

Professor Brendan Wren

London School of Hygiene & Tropical Medicine, UK

This lecture will describe the origin of the production of recombinant glycoconjugate vaccines developed through the functional characterization of an *N*-linked OTase general glycosylation system from the enteric pathogen *Campylobacter jejuni*. It will describe how these basic studies were used to re-constitute the glycosylation system in *E. coli*, which allowed for the first time the production of recombinant glycoproteins through a process termed Protein Glycan Coupling Technology (PGCT). The major application of PGCT is in the construction of affordable recombinant glycoconjugate vaccines. To date, the technology has been used to produce novel recombinant *Campylobacter*, *Shigella*, *Streptococcus pneumoniae*, *Francisella*, *Burkholderia pseudomallei*, *E. coli* and MRSA glycoconjugate vaccines. These have been shown to be protective and some are in clinical trials.

I will describe the applications and limitations of PCGT for the construction of low-cost recombinant glycoconjugate vaccines in three areas; (i) designing novel vaccines against pathogens where no current vaccine exists *(eg Burkholderia pseudomallei)*, (ii) improving existing glycoconjugate vaccines (*eg* pneumococcal vaccine), and (iii) affordable glycoconjugate vaccines for the veterinary market (*eg* poultry, pigs and ruminants).

From a One Health perspective, low-cost glycoconjugate vaccines for animals not only adds to economic prosperity but can reduce animal suffering and the zoonotic transmission of disease to humans. Furthermore, it will reduce usage of antibiotics in the livestock industry and the subsequent spread of antimicrobial resistance.

Successful control of H7N9 influenza in China

Professor Hualan Chen

Harbin Veterinary Research Institute, China

The H7N9 viruses that emerged in China in 2013 were nonpathogenic in chickens but mutated to a highly pathogenic form in early 2017 and caused severe disease outbreaks in chickens. The H7N9 influenza viruses have caused five waves of human infection, with almost half of the total number of human cases (766 of 1,567) being reported in the fifth wave, raising concerns that even more human infections could occur in the sixth wave. In September 2017, an H5/H7 bivalent inactivated vaccine for chickens was introduced, and the H7N9 virus prevalence in poultry after vaccination was successfully controlled based on the large-scale virus surveillance results. More importantly, since the H7N9 vaccine was applied in poultry, only four H7N9 human cases were reported in over two years (three H7N9 human cases were reported between October 1, 2017 and September 30, 2018, and one human case was reported between October 1, 2018 and September 30, 2019), indicating that vaccination of poultry successfully eliminated human infection with H7N9 virus. These facts emphasize that active control of animal disease is extremely important for zoonosis control and human health protection.

Selectively targeting hemagglutinin antigen to chicken antigen presenting cell receptor induces faster and stronger immunity against avian influenza

Angita Shrestha[1],[2], Jean-Remy Sadeyen[1], Deimante Lukosaityte[1], Pengxiang Chang[1], Adrian Smith[2], Marielle Van Hulten[3] and Munir Iqbal[1]

***Affiliations:** **1** The Pirbright Institute, UK; **2** Department of Zoology, University of Oxford, UK; **3** Global Poultry R&D Biologicals Boxmeer, Intervet International BV, MSD Animal Health, Netherlands*

We developed a Target Antigen Delivery Vaccine (TADV) that selectively targets vaccine antigens to chicken antigen presenting cell (APCs) and potentiates immunogenicity of the vaccine. To demonstrate the effectiveness of the TADV, we modified the haemagglutinin (HA) antigen of H9N2 avian influenza virus (AIV) by fusing it with a single chain fragment variable (scFv) antibody specific for chicken CD83 receptors and produced a recombinant soluble trimeric H9HA-CD83scFv protein in insect cells. Vaccination of chickens with adjuvant H9HA-CD83scFv protein induced faster and higher HA antigen-specific antibody titres compared to untargeted H9HA protein vaccine or conventional inactivated virus vaccine. Furthermore, chickens vaccinated with targeted H9HA-CD83scFv vaccine showed reduced H9N2 challenge virus shedding compared to untargeted H9HA. These results suggest that targeting antigens to CD83 receptors could improve the efficacy of poultry vaccines.

Aerosol Delivery of oligodeoxynucleotides containing CpG motifs (CpG-ODN) at hatch against bacterial septicemia in neonatal broiler chickens under field conditions

Susantha Gomis, Kalhari Bandara Goonewardene and Shelly Popowich

Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Canada

Oligodeoxynucleotides (ODN) containing CpG motifs (CpG-ODN) are effective immunostimulatory agents against viral and bacterial diseases. We have recently demonstrated that intrapulmonary (IPL) delivery of CpG-ODN as micro droplets can protect neonatal broiler chickens against lethal Escherichia coli septicemia under laboratory conditions. The objectives of this study were to develop a commercial-scale prototype nebulizer to deliver CpG-ODN as micro-droplets in neonatal broiler chickens at hatch and to study the efficacy of IPL delivery under field conditions in Canada. Neonatal broiler chicks (n= 8,000) received CpG-ODN by the IPL route in the multi-level commercial-scale poultry nebulizer (CSPN) for 30 min and control broiler chicks received distilled water (DW) for 30 min. Broiler chicks were sampled from different locations of the CSPN following nebulization and challenged with a lethal dose of E. coli to study efficacy of CpG-ODN by the IPL delivery. Broiler chicks were protected at a significant level against E. coli challenge following IPL delivery of CpG-ODN in CSPN ($P<0.05$). Chicks treated with CpG-ODN by the IPL route had significantly lower clinical signs and bacterial load ($P<0.05$). We found that IPL delivery of CpG-ODN can induce protective immunity as early as 6 h post-administration and remain effective until day 5 post-treatment. Results of this study confirmed that IPL delivery of CpG-ODN against septicemia in neonatal broilers was industrially feasible and effective under field conditions. This study has demonstrated that CpG-ODN delivery by IPL route can be a promising alternative to antibiotics for inducing protective immunity in chicks during the neonatal life.

Identification of avian influenza viruses subtype H9N2 isolated in 2019 from the backyard and commercial farms in Indonesia

Melina Jonas, Aprilla Sahesty, Theresia Murwijati, Christina Lilis Lestariningsih, Ine Irine, Wahyu Prihartini and Elly Setiawaty

PT Medion Farma Jaya, Indonesia

Low pathogenic avian influenza virus (LPAIV) subtype H9N2 was first detected in Indonesia in 2016. The virus was spread out in various provinces and co-circulating with H5N1. Throughout 2019, we recorded two-hundred-and-seventy cases with clinical signs from both vaccinated and unvaccinated flocks collected from the backyard and commercial farms. Molecular analysis with reverse transcriptase PCR (RT-PCR) and sequencing was conducted to confirm these cases. Positive AIV-H9N2 isolates were inoculated to SPF chickens to observe the pathological signs. RT-PCR showed fifty-four AIV H9N2 positive samples, mostly isolated from layer (46.30%) and breeder (37.04%) farms. The phylogeny of the HA segment of AIV-H9N2 viruses identified in this study appeared to be in the same distinct cluster as 2016-2018 isolates from the previous study. All Indonesian isolates showed high similarity with some Vietnam and China's viruses isolated from chickens and duck in 2012-2014 but formed a different cluster with China's viruses isolated before 2007. There were changes in pathological signs of AIV-H9N2 isolated before and after 2018 when tested in SPF chickens. Haemorrhage occurred less frequent in the trachea and almost none observed in heart samples while these signs were manifested prominently by viruses isolated before 2018. Swollen kidney and intestinal haemorrhage were new pathological changes caused by the recent 2018-2019 viruses. We conclude that the epidemic of AIV-H9N2 continues to affect broilers, layers, breeders and native chickens. Although the newly isolated AIV-H9N2 were placed in the same phylogeny cluster as isolates from 2016-2017, they caused more severe pathological signs on kidney and intestine and less observable tracheal and heart haemorrhage.

Towards edible vaccines for chickens

Dr Kate Sutton[1], Professor Lonneke Vervelde[1], Dr Roger New[2] and Professor Damer Blake[3]

***Affiliations:** **1** Roslin Institute, University of Edinburgh, UK; **2** Proxima Concepts Ltd, UK; **3** Royal Veterinary College, UK*

In this project we tested a novel oil formulation as a vaccine vehicle that targets the mucosal immune system. We have shown the ability of the oil formulation to bind to intestinal epithelial cells and induce both a systemic and mucosal immune response to model antigens in chickens. This vaccine has the potential to be mixed into feed, making it suitable for routine administration to broilers and backyard poultry. This oil formulation is of particular relevance in low and middle income countries where poultry production is rapidly expanding but they have little or no access to cold-chain resources required for current chicken vaccines.

Immunogenicity study of matrix 2 ectodomain proteins displayed on nodavirus-like particles as a universal avian influenza virus vaccine for chickens

Dr Mariatulqabtiah Abdul Razak[1], *Professor Wen Siang Tan*[1], *Dr Kok Lian Ho*[1], *Professor Abdul Rahman Omar*[1] *and Professor Munir Iqbal*[2]

Affiliations: ***1*** *Universiti Putra Malaysia, Malaysia;* ***2*** *The Pirbright Institute, UK*

Highly pathogenic avian influenza (HPAI) H5N1 virus is highly contagious among birds and constantly causes serious outbreaks in parts of Asia and the Middle East. Failure to eradicate its spread and occurrence can lead to severe economic losses and potential health risks in humans. All the commercially available vaccines against H5N1 (inactivated H5N2 vaccine, reverse genetics vaccine, recombinant fowlpox virus vaccine and DNA vaccine), which express the antigenic glycoprotein of avian influenza, haemagglutinin, are unable to completely stop the virus from circulating. The inactivated vaccines against avian influenza virus (AIV) are commonly developed based on circulating low pathogenic avian influenza (LPAI) viruses, hence may not be efficacious against other types of HPAI or LPAI strains. These vaccines are strain-specific and require reconstitution to accommodate any new, mutated circulating viral strains. Development of a universal AIV vaccine targeting all AIV strains is urgently in demand. Our research team has shown a promising protective efficacy of nodavirus-like particles displaying three copies of M2e fragment against human influenza A virus in mice model. We hypothesise that expression of the matrix 2 ectodomain (M2e) proteins from avian influenza viruses of avian origin may confer a universal protection against avian influenza viruses in chickens.

Poster abstracts

1 Surveying Salmonella antigens for use in a bacteriophage-based vaccine enabling homologous and heterologous protection and colonization-inhibition effect in poultry

Angela Makumi[1], Andrea McWhorter[2] and Nicholas Svitek[1]

***Affiliations: 1** International Livestock Research Institute, Kenya; **2** University of Adelaide, Australia*

In the last decade, significant increases in the productivity of modern poultry stocks have been achieved for both the meat and the egg production sectors of the global poultry industry. Regardless of the increase in production trends, the performance of the poultry industry is still low. This is mainly due to low growth rates, high mortality rates, low reproductive rates and poor quality of the product. Bacterial diseases such as Salmonellosis are recognized as an important risk factor in poultry health management. Most poultry farmers are aware of the risk of these infections and their subsequent effect on mortalities, productivity, and profitability. Consequently, farmers spend more money on control and management of bacterial diseases than on any other form of poultry diseases. The past few decades, however, have witnessed a steady increase in the number and diversity of antibiotic-resistant bacteria, rendering some bacterial infections virtually untreatable. The pervasive use of antibiotics for both therapeutic and non-therapeutic purposes is associated with the occurrence of antibiotic-resistant bacteria, due to selective pressure in favour of resistant bacteria. The recommendation to control or prevent Salmonella in poultry flocks include hygiene measures and biosecurity practices which are generally supported by additional interventions, such as the use of antibiotics and inactivated or attenuated vaccines for breeders, which can reduce Salmonella intestinal colonization. The vaccines that are used in the poultry industry include whole pathogen vaccines both live and inactivated which when used play a minor role in controlling salmonellosis in poultry. These vaccines offer short-lived protection against clinical disease and do not elicit a cell-mediated immune response, which is indispensable for the clearance of Salmonella. The objective of the study will entail moving away from whole pathogen vaccines or subunit vaccines into adopting strategies such as incorporating the target antigens as part of a virus (bacteriophage) nanoparticle structure in order produce a multivalent vaccines that will elicit mucosal, systemic immune responses but also control several serovars of Salmonella enterica.

2 Morphological and immunophenotypical evaluations of maturation-associated chicken dendritic cells by wild type and recombinant fowlpox virus infections

Sakinah Yusof[1], Nor Yasmin Abdul Rahaman[1,2], Abdul Rahman Omar[1,2] and Abdul Razak Mariatulqabtiah[1,3]

***Affiliations: 1** Institute of Bioscience, Universiti Putra Malaysia, Malaysia; **2** Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia; **3** Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Malaysia*

Fowlpox is a slow-spreading common viral disease of chickens and turkeys, caused by fowlpox virus (FPV). Infections will lead to lesions on the comb, wattles and other skin areas. Although most birds can survive the infection, it is considered as an important viral disease to combat in commercial poultry farming, as the infections may also resulted in lower production of eggs and reduced body weight. Live vaccines against

FPV have been established since 1920s, while their genome have been manipulated since 1980s to be used as a recombinant vector. Nonetheless, studies on interaction of chicken bone marrow-derived dendritic cells (chBM-DCs) with FPV remains limited. Therefore, this study was aimed to evaluate the response of chBM-DCs upon infections with wild type (WT) FP9 strain of FPV, in comparison to a recombinant FPV carrying the H5 gene of avian influenza virus (rFPV/H5). First, DCs were isolated from chicken femur and cultured for 6 days in the presence of recombinant chicken granulocyte-macrophage colony-stimulating factor (GM-CSF) and recombinant chicken interleukine-4 (IL-4). Then, immunofluorescence staining of FPV-stimulated BM-DCs was performed to detect the presence of CD86 expression marker on the surface, prior to subsequent immunophenotyping using APC-conjugated anti-chicken CD86 and PerCP-conjugated anti-chicken MHC class II. The cultured population showed typical morphology of chBM-DCs with the formation of dendrites, which are the key morphological characteristics that defined as a sign of cell maturation. The result also showed that the infected chBM-DCs expressed enhanced levels of CD86 and MHC class II. In conclusion, this study demonstrates that chBM-DCs were susceptible towards both WT FPV and rFPV/H5 infections, and capable in inducing phenotypic maturation of DCs. This preliminary data may enhance the understanding on FPV and rFPV mechanisms, to improve future development of FPV-based vaccines.

3 Generation of a recombinant chickenized monoclonal antibody against neuraminidase of H9N2 avian influenza virus

Fei Wang[1], Zhimin Wan[1,2], Hongxia Shao[1,2,3], Kun Qian[1,2,3], Jianqiang Ye[1,2,3] and Aijian Qin[1,2,3]

***Affiliations:* 1** *Ministry of Education Key Lab for Avian Preventive Medicine, Yangzhou University, China;* **2** *Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, China;* **3** *Jiangsu Key Lab of Zoonosis, China.*

H9N2 avian influenza viruses (AIVs) have been prevalent in China since the first report in Guangdong province in 1992. It is very difficult to control and causes great economic losses in poultry industry. Based on our previously reported monoclonal antibody (mAb) 1G8 against the neuraminidase (NA) of H9N2 with significant NA inhibitory activity, the gene of fragment of variable region of the monoclonal antibody was sequenced. Then the gene was cloned and fused with the gene of constant region of chicken IgY. The chickenized mAb (rCmAb) was expressed in COS-1 cells and able to secret to supernatant. The significant NA inhibitory activity and neutralizing ability were confirmed in enzyme-linked lectin assay (ELLA) and micro-neutralization (MN) assay. It could be very good passive immunization for H9N2 in the future.

4 Evaluating the effectiveness of vaccines in commercial poultry against Newcastle disease viruses of genotype VI and VII

Muhammad Zubair Shabbir[1], Sameera Akhtar[1], Yi Tang[2], Tahir Yaqub[1], Arfan Ahmad[1], Ghulam Mustafa[1], Muhammad Azhar Alam[1], Diwakar Santhakumar[3], Venugopal Nair[4] and Muhammad Munir[3]

***Affiliations:* 1** *University of Veterinary and Animal Sciences, Pakistan;* **2** *The Pennsylvania State University, PA, USA;* **3** *Department of Biomedical and Life Sciences, Lancaster University, UK;* **4** *The Pirbright Institute, UK*

Newcastle disease virus causes economically devastating disease in avian species around the world. Newcastle disease is enzootic in Pakistan and recurrent outbreaks are frequent in multiple avian species even after continuous and extensive use of vaccines. A number of APMV-1 and pigeon paramyxovirus serotype 1 (PPMV-1) strains have been

isolated and genetically characterized in recent years. However, the impact of recently characterized wild bird-origin APMVs in domestic poultry, and the potency of routinely used vaccines against these novel and genetically diverse viruses remained elusive. Here, we applied next-generation sequencing for unbiased complete genome characterization of APMV-1 and PPMV-1 strains isolated from clinically diseased peacocks (Pavo cristatus) and pigeons (Columba livia), respectively. Global phylodynamics and evolutionary analysis demonstrates Pigeon/ MZS-UVAS-Pak/2014 is clustered into lineage 4 (or genotype VI) and Peacock/MZS-UVAS-Pak/ 2014 into lineage 5 (or genotype VII). The genomes of both isolates encoded for polybasic residues at the fusion protein cleavage motif (112RRQKR↓F117) along with a number of important substitutions in the surface glycoproteins compared with the vaccine strains. Clinico-pathological and vaccine-driven immunological investigations in domesticated chickens indicate that these isolates can potentially transmit between tested avian species, can cause systemic infections, and can induce antibodies that are unable to prevent virus shedding. A noticeable antigenic variation between vaccine strain and wild-originated strain (R-value = 0.26) was also observed while performing cross-HI assay. Collectively, the data from these genomic and biological assessments highlight the potential of wild birds in transmitting APMVs to domesticated chickens. The study also demonstrates that the current vaccine regimens are incapable of providing complete protection against wild bird-origin APMVs and PPMVs.

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Recommended	Recommended	Not recommended	Not recommended	Not supported	Not supported

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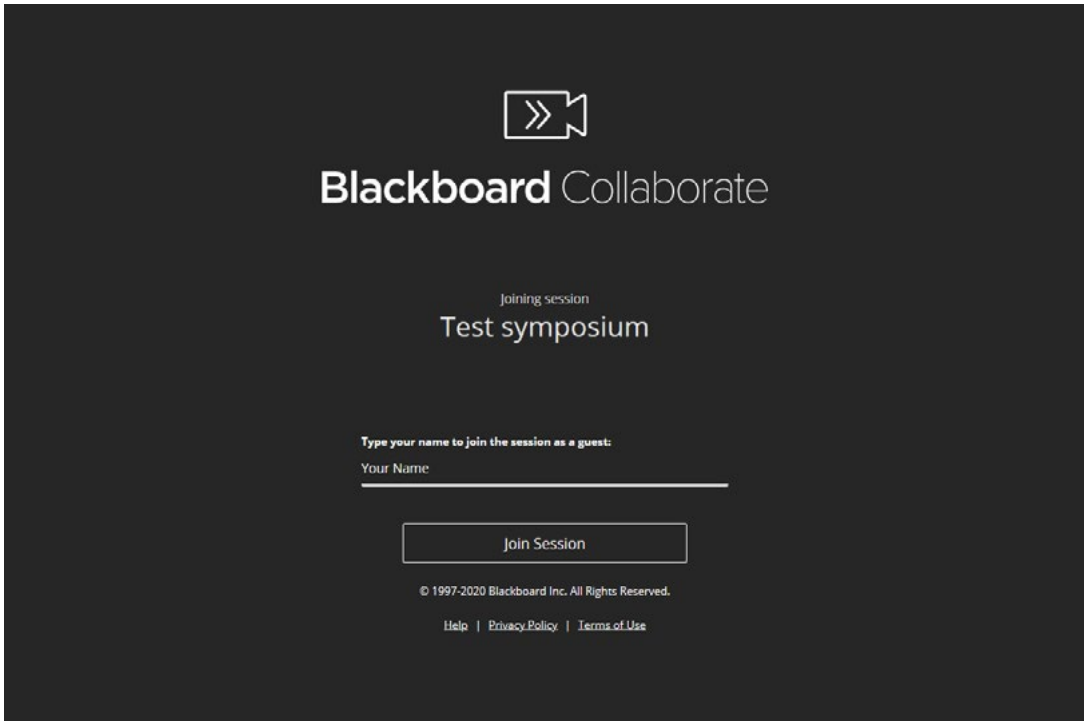

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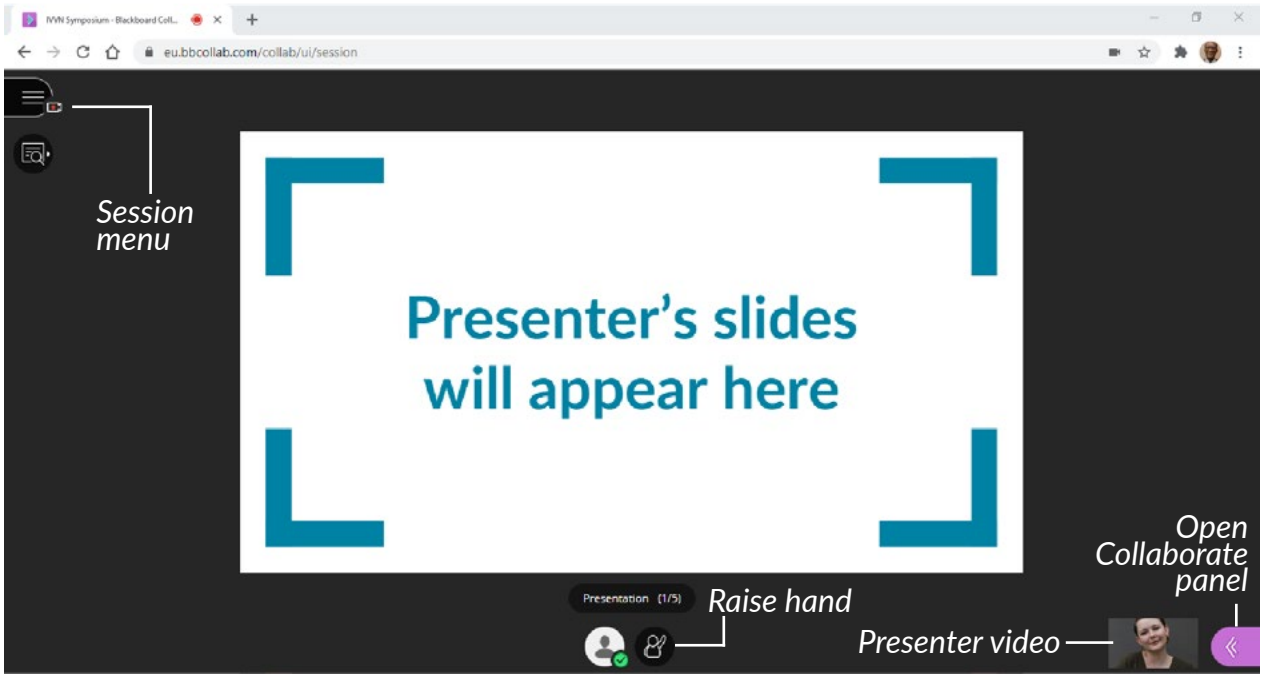

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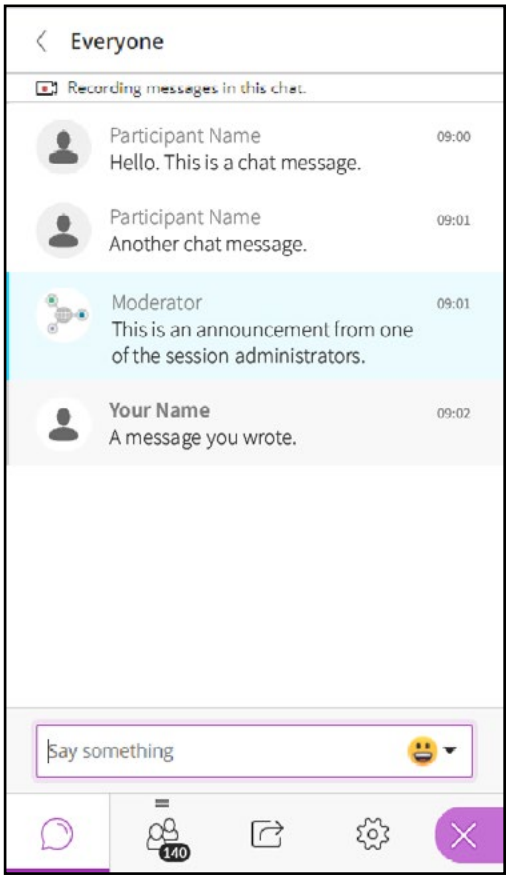

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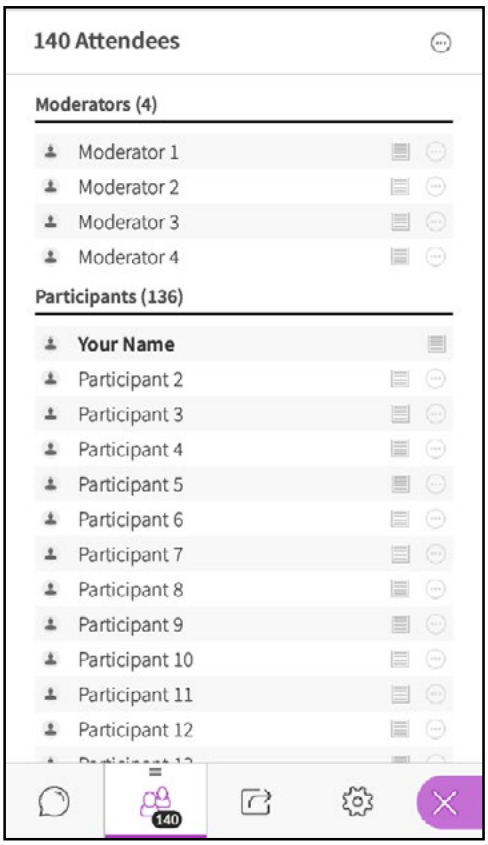

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Poster session

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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.

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