MEETING REPORT

Venoms, poisons and toxins: evolution and impact of amazing molecules

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INTRODUCTION

Venoms 2019, the 6th international meeting on Toxinology at Oxford, co-chaired by Professor David Warrell (University of Oxford, UK) and Dr Edward Rowan (Strathclyde University, UK), successfully accomplished its aims. With over 70 oral and poster presentations this meeting focused largely on venoms. However, topics covering bacterial toxins were also included. One of the aims of the meeting was to allow ample time for discussion and to encourage the formation of new networks as well as re-invigorating established collaborations. More than 100 participants, both established and emerging researchers, came from 22 countries, representing academic institutions, government research organisations, funding bodies, industry, and regional and international charitable organisations. Below is a brief summary of the presentations given at this meeting.

OMICS IN TOXINOLOGY

Dr Edward Rowan chaired the first session, with the inaugural keynote talk by Professor Juan Calvete (Consejo Superior de Investigaciones Científicas, Spain), who showed that proteomic analysis of venoms using Daboia russellii (Russell’s viper) as a model revealed geographical variation despite some proteins being conserved and present in all venoms. Professor Calvete also showed that D. russelli in the Indian subcontinent has a common origin in Pakistan. This was followed by Dr Timothy Jenkins (University of Cambridge, UK) on how treatments should be targeting key toxins rather than whole venom and that antivenoms that contain a known therapeutic content could further the treatment for snakebite. Dr Jenkins also proposed that phage display and droplet microfluidics have the potential to enable ultra-high-throughput discovery and development of antitoxins, while venom gland transcriptomics or other bioinformatic approaches could be crucial to global key-toxin identification. Following this, Miss Lucka Bibic (University of East Anglia, UK), presented work on the P2X4 ion channel to investigate how animal venoms could be used to gain an insight into potential new modulators for ion channels involved in processing pain signals. She suggested that small molecules found in spider venoms had the potential to be explored as analgesics. Dr Cassandra Modahl (National University of Singapore, Singapore) then presented their work on the characterization of venom gland transcriptomes, venom proteomes, and toxin biological activities, using both enzymatic and toxicity assays, for the relative less explored rear-fanged snake species. They found that the venoms of rear-fanged snakes were dominated by either three-finger toxins (3FTxs) or metalloproteinases. The final talk of this session was given by Professor José María Gutiérrez (Universidad de Costa Rica, Costa Rica), who delivered the inaugural Hamish Ogston Foundation keynote lecture on the mechanisms of action of vipherid snake venoms. The key haemorrhagic toxins of the vipherid snake venoms are Zinc-dependent metalloproteinases (SVMPs), which are structurally classified into three classes depending on their venom composition. Proteomic analyses of exudates collected from envenomed tissues have identified previously unknown substrates of SVMPs in the extracellular matrix, which may have implications for the pathogenesis of hemorrhage. Professor Gutiérrez also discussed how low molecular mass metalloproteinase inhibitors are being explored as potential therapeutic tools to complement antivenoms in the treatment of snake venom-induced haemorrhage.

VENOMS AND TOXINS: EVOLUTION, EFFECTS AND FUNCTIONS

Aspects of evolution, effects and functions of venoms and toxins were the main topics in Session 2, which was...
chaired by Professor Dietrich Mebs. **Dr Denise Tambourgi** from the Butantan Institute in São Paulo, Brazil commenced this session and spoke on the function of C-SVMP and its role in activating the complement system leading to an increase in the inflammatory process and discussed the role snake venom metalloproteinases play in the activation of the complement system, such as inducing the formation of anaphylatoxin. Applying compstatin, an inhibitor of the complement system was shown to control the inflammatory response in snakebite envenoming. In his presentation, **Dr Sebastien Dutertre** from the CNRS in Montpellier, France, explored the predatory and defensive functions shaping venom evolution. He demonstrated that carnivorous cone snails (*Conus* spp.) are able to apply different venom types either for predation or defence depending on the stimulus. **Dr Timothy Jackson** from the University of Melbourne, Australia, suggested that understanding snake venom evolution and diversity including the ecological background is essential for designing antivenoms and will help to tackle the current crisis of snakebite envenoming. The inflammatory response following snakebite must be considered as a systemic pathology, said **Miss Chloe Evans** from the Liverpool School of Tropical Medicine. She reported results of her studies on medically important snakes of Sub-Saharan Africa, which caused elevation of inflammatory markers in mice and advocated further clinical research on this topic.

At the end of this session, **Professor Alan Harvey** (Strathclyde University, UK) was presented with a Life-time Achievement award for his extraordinary contributions to the field of toxinology (publishing on average 6 articles per year since 1974), in particular for the discovery of dendrotoxins and multiple toxins as specific research tools for neuropharmacology, and his leading role in advancing the mission of the International Society on Toxinology (Figure 1).

**DRUGS FROM TOXINS**

Professor Juan Calvete chaired the 3rd session of the meeting which focused on the development of drugs from venoms. **Professor Alan Harvey** opened this session with his keynote speech on the various “dos and don’ts” about using venom toxins for drug discovery. He pointed out that despite considerable and continued interest in this area, the number of toxin-based drugs is small, and argued that this might be because: i) most toxinology research is carried out in academic laboratories, which concentrate more on research than on the development of new therapeutics; and ii) the academic laboratories appear to lack the range of skills needed for successful drug discovery and development projects. Following this, **Professor Glenn King** (The University of Queensland, Australia) spoke about the development of venom peptide inhibitors of acid-sensing ion channel 1a for treating ischemic injuries of the heart and brain, including stroke and myocardial infarction. **Dr Elaine Fitches** (Durham University, UK) reported on a novel spider venom based biopesticide for target-specific control of the small hive beetle, which is a serious pest of European honey bee. Using ω-hexatoxin-Hv1a and Hv1a/GNA FPs

![Figure 1](image_url): Professor Alan Harvey received a life-time achievement award at Venoms and Toxins 2020.
spider toxins as biopesticides, they developed a method of oral delivery of insecticidal peptides to their site of action via fusion to a “carrier” protein that directs transport of an attached toxin across the insect gut to the circulatory system. Dr Keith Miller (Sheffield Hallam University, UK) then showed that changes in charge and hydrophobicity on venom-derived Smp43 and Smp43-modified peptides alter antimicrobial activity, but also haemolytic and cytotoxic activity to improve therapeutic index. Following this, Professor Jan Tytgat and his colleagues, Drs Steve Peigneur (KU Leuven, Belgium) and Ernesto Lopes Pinheiro Junior (University of São Paulo, Brazil), presented their work on potentially antitumor and insecticidal peptides from snake and wasp venoms, respectively. Their work largely focused on venom proteins with ion-channel blocking properties, such as collinein-1, which is a serine protease isolated from the venom of Crotalus durissus collilineatus. The same protein, when PEGylated, exhibits a promising application for specific haemostatic conditions, due to its fibrinogenolytic activity. They also showed that γ-PMTX, isolated from Cyphononyx fulvognathus venom, a novel member of the PMTX family of small (13-15 residue) – non-disulfide bridge peptides, acts by slowing down channel inactivation. The final presentation of this session was by Dr Aneesh Karatt-Vellatt (IONTAS Ltd, Cambridge, UK), who presented work on KnotBodies, in which venom derived mini-proteins were fused to antibody CDR loops to treat ion channel mediated pathologies.

INSPIRATION, INNOVATION AND INVESTMENT IN SNAKE-BITE RESEARCH

Recently, several regional and international organisations, including the World Health Organisation (WHO), the Wellcome Trust and the Hamish Ogston Foundation (HOF) have committed considerable funding to advance research on snake-bite. The aim of this session, which was chaired by Professor David Warrell, was to invite some of these key organisations to share their vision and to layout their ideas about their funding strategies.

The first talk in this session was jointly given by Drs Bernadette Abela-Ridder and David Williams from the WHO, which is seeking US$ 136.76 million to support snake-bite prevention control and projects from 2019-2030 (WHO Neglected Tropical Diseases/Snakebites, 2019). They outlined their current strategies and the roadmap to prevent and control snakebite, as well as other neglected tropical diseases around the world and their ‘Patient-centric approach’ providing pharmaceutical, psychological and health system support. They pointed out that the WHO also works with academia and pharmaceutical sectors to increase their reach in achieving their goal to halve the numbers of snakebite deaths and cases of disability (which have reached the level of a global epidemic) by the year 2030. They are also working on empowering communities to better prevent snake-bite, pre-hospital care, etc. The WHO are not only supporting human welfare but, from a ‘One Health’ perspective, are also concerned about the welfare of livestock. Companion animals are major assets for poor village families, and the loss of an animal can be a major financial burden. Under the WHO’s community engagement programme, the local communities are encouraged to join forces with local community groups, civil society activists and a range of collaborating partners to improve the prevention of snakebite (e.g., by using protective foot and hand wear). Professor Mike Turner from The Wellcome Trust (UK) then spoke about the Trust’s snake-bite initiative. He started by introducing the activities of the Wellcome Trust, which are primarily aimed at supporting biomedical research. The Trust has reserved funds for strategic initiatives to achieve specific objectives and has allocated £80 million for their snake-bite initiative over a fixed-term of seven years, with deliverables aligned with those of the WHO. For example, these funds will be used to: i) modernise antivenom production facilities; ii) develop next generation treatments that have not been applied to the snake-bite, such as phopholipase A, inhibitors, which have been developed for other diseases and have some safety data in humans; and iii) develop policy-oriented global and regional health systems. Following this Professors Calvete and Gutiérrez presented aspirations of the Global Snakebite Initiative (GSI), which is a non-profit charitable organisation, formed in 2008 in Australia. The primary aims of GSI include community education, clinical reporting, improving access to drugs, worker education and training and supporting research. GSI’s historical goal has been for the snakebite to be fully recognised as a global public health emergency. However, the key turning point in the GSI’s initiative came in 2016 when they with global partners, such as HAI, MSF, and a consortium of ~25 diplomatic missions in Geneva, were able to put together a side event at the World Health Assembly (WHA), which received the attention of key stakeholders from around the world and the WHO. As a result of this effort, in 2018, snakebite was regarded as a category A disease in WHO’s register of neglected tropical diseases (NTDs). It is anticipated that this will set the foundations for a global collaboration between countries to fight snakebite. Dr Cathy Roth (Department for International Development - DfID, UK) explained DfID’s wider activities, their interest in snakebite, how they are pursuing this interest and the realities and challenges of taking science into application. Product development partnership is one of the areas in which DfID invests, along with the pharma industry and academic researchers, which includes the development of snake antivenoms. The key reasons for DfID’s interest in this area are based on help and the economic human capital perspectives, and so they are investing £9.2 million in snake antivenom research. For example, DfID is supporting the Liverpool School of Tropical Medicine to develop (ideally) a universal snake antivenom, as well as regional antivenoms. Dr Michael Vaughan (Hamish Ogston Foundation, UK) then described HOF’s initiatives covering Health, Heritage and Music. Under “Health”, they are investing £3.4 million in antivenom trials in India, Vietnam and Myanmar. HOF regards snake-bite as a very fertile area for research, innovation and discoveries. HOF also funded the first of the foundation’s lectures at the Oxford venoms meeting and prizes for the best poster presentations. Dr Benjamin Waldmann represented Health Action International (HAI, Netherlands). HAI stepped in at a very crucial stage of the strategy of GSI to achieve the NTD recognition of snake-bite at the WHO, and their knowledge of the diplomacy at Geneva (WHA) was absolutely essential in achieving this objective. HAI works around health system advocacy partnerships, conducts research and advocacy,
leads incident support, shapes programmes around access to essential medicine (including antivenoms) and medicine generally, community education and capacity development in low-to-middle income countries. HAI encourages multi-stakeholder dialogue for practical implementation of their programme. Kenya, Uganda, Zambia, Burundi, and South Sudan are some of the countries where they are currently working to develop improved medical practices, involving youth and the civil society. Dr Tamar Gosh was the final speaker of this session, presenting on behalf of the Royal Society of Tropical Medicine and Hygiene (RSTMH, UK).

Like many other organisations, they are focused on eradicating disease, as well as saving and improving lives. She introduced RSTMH’s interest in snakebite, scientific publications and small funding opportunities in the snakebite research area, in particular those at the beginning of 2020. This concluded with discussion and networking dinner in the beautiful settings of St Hilda’s College.

NEWLY RECOGNISED VENOMOUS TEXA

The second day began with a keynote address by Professor Dietrich Mebs (University of Frankfurt, Germany) who discussed the acquisition of toxins by animals, which could be achieved not only by gene expression and complex metabolic pathways, but also through the uptake, accumulation and storing of toxic metabolites produced by other organisms, such as microbes, plants and other animals. He pointed out that it was difficult to estimate and compare the metabolic cost and efficiency of the two methods of acquiring toxins. Nevertheless, resistance at a molecular level (receptors or ion channels) would need to be developed when sequestering toxins, which may eventually outweigh the benefits of saving metabolic energy. Professor Mebs concluded that both ways of acquiring toxicity, synthesis or sequestration, have proved equally successful during evolution, leading to a huge diversity of toxic compounds. Following this, Dr Ronald Jenner (London Natural History Museum, UK) spoke on the evolution of centipede venom composition and discussed their work in which they conducted the first ever comparative proteotranscriptomic analyses of centipede venom composition including representatives of all five orders of centipedes (Arthropoda: Chilopoda). They concluded that there was no such thing as ‘typical centipede venom’, and that centipede venom components are unique to each of the five centipede orders. Thereafter, Dr Christopher Lynch from Professor Bhattacharya’s Laboratory at the University of Oxford (UK) presented their work on tick evisons (Evasin-3), which are proteins derived from the salivary glands of ticks and display anti-chemokine activity. Tick salvia contains a wealth of molecules that modulate both the immune and haemostatic systems of the host. Their work on yeast surface display identified novel chemokine-binding proteins from tick salivary transcriptomes. Mr James Dobson (The University of Queensland, Australia) then presented his work on the coagulototoxic activity of anguimorph lizard (Heloderma and Varanus species) venoms. Varanid lizard venoms produced varying anticoagulant activity via the destruction of fibrinogen, while Heloderma venoms produce mild procoagulant activity produced by activating FXII and FVII. Mr Jonas Krämer (University of Cologne, Germany) presented on the development of a venom collection method from the pseudoscorpion Chelifer cancroides and structural elucidation of its venom components. Mass spectrometry revealed nearly 30 distinct ion signals from the venom from two specimens, which were almost identical. Using the top down proteomics approach in combination with transcriptomic data, enabled them to identify venom compounds that show sequence similarity to Megicin, an antimicrobial peptide known from the venom of the scorpion Mesobuthus gibbosus. Then, Mr Bjorn Marcus von Reumont (LOEWE Centre for Translational Biodiversity Genomics, Germany) spoke on the venom system and the genomic processes of toxin evolution in robber flies (Asilidae, Diptera). They used a broad comparative toxicogenomic analysis on several species to gain an insight into the venom system of robber flies. From 30 identified predominantly venom proteins in the non-asilid genomes, 15 highly expressed venom proteins appear to be unique to robber flies. Their findings underpinned the significance of further genomic studies to cover more neglected lineages of venomous taxa and to understand the importance of orphan genes as possible drivers for venom evolution. Following the refreshment break, Professor Paul Long (King’s College London, UK) spoke about the presence of toxin-like peptides from Polypodium hydridiforme, a cnidarian adapted to parasitism of developing oocytes of acipenseriform fish (sturgeons and paddlefishes), which they identified using genomics, transcriptomics and proteomics approaches. Based on their studies they concluded that Polypodium is a venous cnidarian and that Polypodium expresses has more toxins than free-living relatives. They also concluded that the toxins have been co-opted for an endoparasitic lifestyle. Dr Gary Bucciarelli (University of California, LA, USA) then gave a presentation on assessing rangewide variation of toxin defenses in a poisonous amphibian, newt (genus Taricha). They characterized the spatial and temporal patterns of newt tetrodotoxin (TTX) from nearly 50 populations across California, assessed the magnitude of variation across space and time, and determined the degree to which TTX within populations matches known predator resistance. Their results suggested that a co-evolutionary model does not explain the evolution of TTX in newts. Furthermore, it was still not well understood how newts have acquired TTX.

BACTERIAL TOXINS

The sixth session was chaired by Professor Jan Tytgat, which focused on toxins of bacterial origin, and was opened by Professor Oliver Dolly (City University Dublin, Ireland) who presented his laboratory’s work on the mechanisms of action of chimeric botulinum neurotoxin on TRP/V1 and /A1 channels in sensory neurons. Using immuno-cytotoxic labelling, they demonstrated that TNFα augments the surface content of transient receptor potential (TRP) vallindio 1 (TRPV1) and ankyrin 1 (TRPA1) transducing channels on cultured dorsal root ganglion (DRG) neurons, which, in turn, enhances the electrophysiological and functional responses of the latter to their specific agonists. They produced a chimeric protein, consisting of the protease light chain (LC), botulinum neurotoxin (BoNT) serotype E fused to full-length BoNT/A (LC/E-BoNT/A), and found that LC/E-BoNT/A abolishes the TNFα-dependent
augmented surface trafficking of TRPV1/A1. Professor Dolly proposed that the observed inhibition by LC/E-BoNT/A of its action in vitro could contribute to its potential alleviation of pain. Drs John Barr and Suzanne Kalb from Centers for Disease Control and Prevention, USA, then presented their work on anthrax and Botulinum toxins. Dr Barr presented on characterization of anthrax toxins during the course of inhalation infection in non-human primate models and showed how the ratios of toxin levels (LF/LTx, LF/EF, EF/ETx) indicate stage of infection, which change throughout the course of bacterial infection. The LF/EF ratios were high in early infection, while LTx/LF and ETx/EF levels were low in early infections but were almost all in the complex form (LTX and ETX) at the time of death. These data have the potential for predicting treatment failures, and provided guidance for development of point-of-care diagnostics and advanced therapeutics. Dr Suzanne Kalb then presented their work on the development of a mass spectrometric method for the detection and characterization of botulinum neurotoxins (BoNT), toxin subtypes/variants beyond the serotype level, and to discover novel BoNTs and their enzymatic activity. They were also able to apply this method to identify enzymatic activity of a novel protein from S. enterococcus whose genetic sequence was similar to that of the bont gene. Dr Celia Carlini (Pontificia Universidade Católica do Rio Grande do Sul, Brazil) then presented on the non-enzymatic biological properties of ureases in relation to pathogenesis, which are multifunctional, highly-conserved proteins which differ in the number of subunits and possess several non-enzymatic biological properties. Considering the homology among microbial ureases it is expected that many of them display the non-enzymatic biological properties described for Helicobacter pylori and Proteus mirabilis ureases. The non-enzymatic properties of ureases probably add or synergize with their enzyme activity in the role of these proteins as virulence factors of pathogenic microorganisms. Dr Carlini proposed that new urease inhibitors with therapeutic value, which so far have been directed solely towards the enzyme’s active site, should also take into consideration their non-enzymatic properties to be fully effective.

MONOCLONAL ANTIVENOMS

Dr Andreas Laustsen (Technical University of Denmark, Denmark) presented an overview of the possibilities for designing cocktails of recombinant human antibodies that could offer protection against relevant toxins from a variety of venoms. The idea is to select antibodies of broad coverage, effective in the neutralization of toxins present in different venoms, hence providing wide therapeutic possibilities. The long term goal is to design mixtures of recombinant antibodies that would neutralize a variety of toxins present in medically-relevant venoms. Following this, Dr Patrick Waters (University of Oxford, UK) described the occurrence of antibodies against dendrotoxins from mamba snake venoms in a group of people who work as snake handlers. Laboratory tests to screen for these antibodies have been developed and this offers the possibility of producing recombinant antibodies against dendrotoxins. Miss Charlotte Dawson (Liverpool School of Tropical Medicine, UK) then described the development of multi-toxin epitope strings, synthesized on the basis of the identification of relevant epitopes in toxins from medically-relevant African snake venoms. The design of the toxin multi-epitope strings was centered on toxins which cause tissue necrosis. This offers an alternative for the design of antigens which would improve the antibody titers in antivenoms against necrotizing components, enabling a broad spectrum of efficacy. Finally, Miss Nessrin Alomran (Liverpool School of Tropical Medicine, UK) explored the design of pathology-centred antivenoms, by immunizing sheep with a mixture of hemotoxic snake venoms. This is a different approach from the one traditionally used which is based on a geographically-based design. The antivenoms generated were effective in the neutralization of hemotoxic effects of a wide variety of snake venoms, demonstrating the value of this strategy for antivenom design. The presentations promoted a discussion on the various topics presented. The researchers and groups that participated in this session provided evidence on the possibilities of using novel technological approaches, some of them based on recombinant DNA technology, to generate antivenoms of higher efficacy against a variety of animal venoms.

CLINICAL TOXINOLOGY

The final session of the conference on clinical toxicology was led by Dr David Williams from the WHO/ University of Melbourne (Australia). The first talk in this session was given by Dr Naira Ayvazyan (Orbeli Institute of Physiology of the National Academy of Sciences, Armenia) who discussed snakebite envenoming and the lack of availability of appropriate antivenoms specific for endemic vipers in Armenia. They are currently developing monospecific antivenom for envenoming by Levantine vipers (Macrivipera lebetina) and plan to develop also polyclonal antivenom for the other vipers species of Armenia. Dr Ayvazyan concluded that the severity of envenomation and the antivenom efficacy for human could be defined also by the blood group of the patient. Professor Abdulnasali Nasidi (University of Africa, Nigeria) then spoke about the work of EchiTabStudy Group (ESG), Nigeria, to tackle the widespread mortality and morbidity caused by the carpet vipers (Echis ocellatus complex) across Nigeria and the West Africa, and to develop a monovalent antivenom. The carpet viper is responsible for over 90% of bites and 65% of snake bites in the region. Two whole IgG antivenoms were produced in the collaborative work: Monospecific Echis ocellatus/romani (“EchiTab G” in collaboration with MicroPharm, UK), and Polyspecific Echis ocellatus/romani-Bitis arietans-Naja nigricollis (“EchiTab Plus ICP” in collaboration with Instituto Clodomiro Picado Costa Rica). He reported that the use of these antivenoms had reduced case-fatality to less than 1%, from well above 40%. Based on their longstanding work the Nigerian Government has appointed the EchiTab Study group to establish an Antivenom manufacturing facility in Nigeria. Another clinical presentation was given by Professor Aniruddha Ghose (Chittagong Medical College & Hospital, Bangladesh) about the Green pit viper bite, causing considerable morbidity and potential threat to victims’ health due to coagulopathy and substantial local tissue swelling. He presented results of a yearlong study of Green pit viper victims at a tertiary hospital in southeast Bangladesh. Overall, their study gave a clinical picture of Green pit viper victims, demonstrating
it as a cause of significant morbidity having a potential for mortality and pointed out that effective antivenom against the Green pit viper venom, the commonest cause of venomous snakebite in Bangladesh, is still unavailable. Dr Andrew Watt (University of Melbourne, Australia) then spoke about their PNG Snakebite Partnership (launched in 2018, between the University of Melbourne, the National Department of Health (PNG) the Australian Government, Seqirus Pty Ltd, and the Charles Campbell Toxinology Centre at the university of Papua New Guinea), an antivenom distribution programme to tackle snakebite across Papua New Guinea, which has one of the highest region-specific snakebite rates in the world. Under this programme, 600 vials of antivenoms against snake and stonefish venoms (donated by Seqirus) have been distributed to health centres across PNG each year and vital incidence and clinical data are being collected on each suspected snakebite patient. This study will conclude in 2020.

The conference concluded with Professor David Warrell (University of Oxford, UK) giving a presentation on the Australian DFAT-GPFD Myanmar Snakebite Project. This project was enabled by a grant from the Australian Government to the University of Adelaide in 2015 for improving the health outcomes for snakebite patients in Myanmar. This project was run in collaboration with Government of Myanmar and ended in October 2018. Snakebite in Myanmar is dominated by the Eastern Russell’s Viper (*Daboia siamensis*), causing 70% envenoming and deaths, with e farmers being at a particular risk. Specific aims of the project included: improving quantity and quality of antivenom production by Burma Pharmaceutical Industry (BPI); increasing availability of antivenom to health centres especially in rural regions; and optimising management of snake-bite patients at the community level. In addition, seminars and training was also offered to the medical students and staffs. The key net outcomes of this project included a radical improvement in the quantity and quality of local antivenoms, removing the need to import antivenoms, and the optimisation of management of snake-bite patients at the community level. This work also resulted in numerous scientific publications (for example, Alfred et al, 2019; Sai Sein Lin Oo et al, in preparation).

A number of poster prizes were also awarded at this meeting to young researchers, which were sponsored by the Hamish Ogston Foundation. The meeting ended with closing remark from Professor Warrell, thanking all participants and sponsors of the meeting and announcing the likely dates of the next meeting as 25-26th August 2020.

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