17th MEDICAL BIODEFENSE CONFERENCE

28 Sep - 1 Oct 2021, Munich, Germany

Medical Biodefense Conference

Honour the Past, Embrace the Future

SCIENTIFIC PROGRAM & ABSTRACTS

organized by
Bundeswehr Institute of Microbiology

Program at a glance

Click on session of interest to jump to the corresponding abstracts!

Wednesday, September 29

Audimax	Garden Hall
A Opening session	
B Outbreak and epidemic management	
C Information for action: Surveillance and epidemiology	**D** Four- and eight-legged warriors
E Phylogenetics and genetic diversity	**F** Case reports and educational session

Thursday, September 30

Audimax	Garden Hall
G Last minute highlights: Aspects of zoonotic infections - from detection to therapy	**H** A tight match: host vs. pathogen
I New tools for stationary and mobile diagnostics (Part 1 of 2)	**J** The dose makes the poison: An update on the detection of biological toxins
K New tools for stationary and mobile diagnostics (Part 2 of 2)	**L** Dual-use-research of concern
M Antimicrobials and vaccines	**N** International biosecurity

Friday, October 1

Audimax	Garden Hall
O Bacteriophages against highly pathogenic and MDR bacteria: from diagnostics to therapy (Part 1 of 2)	
P Bacteriophages against highly pathogenic and MDR bacteria: from diagnostics to therapy (Part 2 of 2)	
Q Poster awards and farewell	

Audimax / 09:00 ... 10:30

A

Opening session

Chairs: Roman Wölfel (DEU) and Lars Schaade (DEU)

Session summary

Presentations are online (▭) or on-site (👤) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
A1 👤	**Bernd Mattiesen** Munich, DEU	Welcome Note by the Director Science and Deputy Commander of the Bundeswehr Medical Academy	10+0 min
A2 👤	**Roman Wölfel** Munich, DEU	Welcome Note by the Conference Chair and Institute Director of the Bundeswehr Institute of Microbiology	25+0 min
A3 👤	**Andrew D. Green** Birmingham, GBR	For Whom the Bell Tolls: The Global Challenges posed by New and Emerging Infectious Diseases	55+0 min

A3

For Whom the Bell Tolls: The Global Challenges posed by New and Emerging Infectious Diseases

Andrew D. Green
Royal Centre for Defence Medicine, Birmingham, United Kingdom

Transmissible infectious diseases with the ability to spread rapidly have always been able to cause outbreaks. The advent of rapid international travel in the 20th century enabled what were once localised public health issues to more commonly become incidents that extend beyond national borders, and develop in global pandemics.

The challenges posed by novel infectious diseases include:

- initial recognition in a population never previously exposed to a specific disease
- developing a clinical case definition to allow accurate descriptive epidemiology of the disease in the community
- initial identification and then laboratory diagnosis of a novel pathogen
- establishing evidence-based management protocols for patient management for a disease for which there is no past experience
- identification of routes of transmission to allow appropriate public health interventions to be made
- development of therapeutic interventions for treatment of cases, to prevent severe effects of the disease, to reduce transmission, or prevent infection of susceptible individuals (including immunisation)
- surveillance of both the disease and the pathogen in populations, including animal reservoirs where relevant

The consequences of a large-scale outbreak extend beyond the direct impact on health of the human population, and may have economic effects both as a direct result of illness and second order effects related to control measures used. There may be impacts on social well-being including civil disruption, damage to national and local infrastructure, and conflict resulting from disruption to national and international security.

Recognition of the potential impact of such events related to novel infections were first articulated in the early 1990s, and a series of proposals for national and international preparedness made at regular intervals since then. Most were not enacted due to competing demands from immediate economic and political priorities, since the timing of a global pandemic could not be predicted with certainty. Until the arrival of 2020.

This talk aims to provide a background to the pandemic of COVID-19, and illustrate the importance of investment in scientific and medical expertise to provide future resilience for future pandemics. It will also outline the geo-political significance of such events, and describe why both military and civilian experts are essential and have complimentary roles.

Audimax / 11:00 ... 12:30

B

Outbreak and epidemic management

Chairs: Gelimer Genzel (DEU) and Ines Mergler (DEU)

Session summary

Presentations are online (▭) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
B1 🧍	**Ines Mergler** Berlin, DEU	Strengthening the United Nations Secretary-General's Mechanism (UNSGM) during times of COVID-19 pandemic-related restrictions	20+5 min
B2 🧍	**Jonathan W. Ashcroft** London, GBR	The British Army's approach to science and technology in an ever-changing world	12+3 min
B3 🧍	**Julia K. Burr** Alexandria, VA, USA	Requirements for the Implementation of COVID-19 Control Measures Given Prevailing Rates of Vaccine Compliance	12+3 min
B4 🧍	**Benjamin Queyriaux** Munich, DEU	Strategic autonomy (self-sufficiency) of Europe for supply of CBRN medical counter-measures	12+3 min
B5 🧍	**Ralf Wörl** Elmshorn, DEU	Safe containments, tailored to the application – up-scaling, modification, integration, combination and complete enclosures	12+3 min

B1

Strengthening the United Nations Secretary-General's Mechanism (UNSGM) during times of COVID-19 pandemic-related restrictions

Ines Mergler, Anja Blasse, Claudia Borawski, Maria Hecht, and Stefan Kloth
Robert Koch Institute, Centre for International Health Protection (ZIG), Berlin, Germany

With a global pandemic ongoing, many international projects have been forced into new ways of thinking, giving an impetus to virtual initiatives. The Robert Koch Institute (RKI) has built on this opportunity to develop innovative ways for strengthening the so-called United Nations Secretary-General's Mechanism (UNSGM) for the investigation of alleged use of chemical, biological or toxin weapons.

Since 2014 the RKI strengthens the UNSGM by conducting trainings for qualified experts and laboratories that are nominated for a possible involvement in potential future missions to investigate the use of biological weapons. As part of their current project, an RKI team from the Centre for International Health Protection (ZIG) has recently - and partly due to the COVID-19 pandemic - focused on virtual activities that aim to identify and meet existing capacity needs related to the UNSGM. Two major milestones of those efforts are a virtual tabletop exercise (TTX; held in November 2020) and an e-learning module (to be published in summer 2021). As part of a full-scale capstone exercise, during which an entire investigation mission will be simulated as realistic as possible, the RKI developed a virtual TTX that represented the initial mission planning phase of the exercise. Initially planned as an in-person event, the new format presented the first virtual TTX in the context of the UNSGM. A web-conference tool allowed participants from several time zones to discuss during the TTX, while the mission planning was taking place on an innovative web-platform which also delivered injects for the experts. The subsequent field exercise will take place in 2022 in Berlin. Taking into consideration the broad geographical dispersion of UNSGM experts, also more established formats such as e-learning present a possibility to make training available to a large number of experts and in a self-paced manner. With this in mind, the RKI has developed an e-learning module that introduces qualified UNSGM experts to the topic of personal protective equipment (PPE). In addition, two complementary modules about risk assessment as well as donning and doffing of PPE are planned. All modules will be hosted by the United Nations Office for Disarmament Affairs. Against the backdrop of the COVID-19 pandemic, virtual formats allowed a continuation of activities during times of travel restrictions, but might also be a promising supplementary method to strengthen the UNSGM in the future.

B2

The British Army's approach to science and technology in an ever-changing world

Jonathan W. Ashcroft
Ministry of Defence , Army - Defence Science & Technology, London, United Kingdom

We find ourselves in uncertain and turbulent times. The world of today is increasingly complex, with new competitors challenging the established international order. The pace of change is accelerating with new scientific fields emerging, new technologies being developed and adopted faster than ever, and new analysis techniques capable of digesting mountains of data are enabling rapid command and control support. Science and technology (S&T) are now themselves viewed as theatres of strategic competition. New adversaries have emerged, alongside traditional threats, actively seeking to undermine our democracy and society, through both traditional and non-traditional approaches to warfare and geopolitical competition. The use of CBRN-related capabilities as well as the growing exploitation of the digital sphere to pursue foreign and domestic agendas highlights this point. Further complicating this terrain has been the rise of terrorist groups, non-state actors and proxies which can access technologies and weapons far more sophisticated than before in pursuit of their parochial interests.

The natural environment is also challenging us in complex and impactful ways (including the SARS-CoV-2 pandemic and climate change) necessitating that we need to adapt and modify our approaches and behaviours accordingly. The British Army (and UK Defence collectively) recognises that we must change how we invest in, and develop, capabilities to avoid both falling behind our adversaries and in step with a changing environmental landscape. We must fuse this insight, together with policy implications (including ethical considerations), to ensure we identify and integrate emerging technologies into generation-after-next capabilities for our armed forces and deliver the capability outcomes we need. The British Army has recognised that it must act differently in order to meet these challenges and to realise our ambition of becoming an integrated high-tech armed force that is able to deter, prevent and if required, tackle, credible threats. To enable this, UK Defence as a whole will focus our efforts on our most significant and enduring capability challenges, where emerging technologies can give the UK a decisive edge in the future. We will balance this pull with a concurrent technology push, to nurture specific promising technologies. It is through the thoughtful and timely development and application of S&T the we believe we can seize opportunities and pre-empt future threats.

B3

Requirements for the Implementation of COVID-19 Control Measures Given Prevailing Rates of Vaccine Compliance

Julia K. Burr[1], Robert L. Cubeta[2], Lucas A. Laviolet[1], and Sean M. Oxford[3]
1- Institute for Defense Analyses, Strategy, Forces and Resources Division, Alexandria, VA, USA; 2- Institute for Defense Analyses, Strategy,Forces and Resources Division, Alexandria, Virginia, USA; 3- Institute for Defense Analyses, Strategy, forces, and Resources Division, Alexandria, Virginia, USA

In this presentation, we describe our analytical approach to determining the requirements and opportunities for using various measures to control outbreaks of COVID-19, assuming the primary objective is to reduce the average number of infections caused by a contagious individual in any given outbreak (R) to below one. We establish relationships between a set of parameters describing characteristics of both disease and response measures, from which we are able to determine the circumstances in which outbreak control measures could be effective. In particular, we explore how the requirements for various control measures, such as isolation and quarantine, can vary given different rates of vaccine compliance in a population.

We begin by developing a profile of mean individual COVID-19 transmission over time that accounts for both pre-symptomatic and asymptomatic transmission. We then use that profile to determine the point in time, relative to both exposure and mean symptom onset, when an individual would, on average, transmit COVID-19 to one other person. Next, we assess the requirements for implementing control measures such that they would, collectively and on average, stop transmission at or before that point in time. We consider a layered approach, beginning with vaccination and adding isolation, triggered by either symptom onset or as a result of diagnostic testing, and finally quarantine.

Our work shows that COVID-19 outbreaks cannot be controlled solely through isolation of symptomatic individuals, given the high transmissibility of COVID-19 combined with asymptomatic and pre-symptomatic transmission. Vaccines can overcome this challenge if they are sufficiently effective, and if compliance rates are sufficiently high. Yet if vaccine compliance rates remain low in certain regions, or if the effectiveness of vaccines is compromised by the emergence of variants, transmission of COVID-19 may continue or even increase. Should that be the case, our assessment shows that

- assuming prompt isolation of symptomatic individuals continues

- implementation of quarantine and/or population-wide diagnostic testing can cause an outbreak to wane.

While more burdensome than vaccination and isolation, these measures can be effective if limited to quarantine of readily identifiable household contacts, or screening tests administered weekly. Screening in particular may avoid the disruption that comes from quarantine and other forms of restriction of movement.

B4

Strategic autonomy (self-sufficiency) of Europe for supply of CBRN medical counter-measures

Benjamin Queyriaux
HIPS Agency, Munich, Germany

Background: To prevent or treat infectious diseases or intoxications, including CBRN medical counter-measures, health care uses mainly antimicrobials and antidotes. These medications have a complex production process from the synthesis of the Active Pharmaceutical Ingredient (API) to the manufacturing of pills or injectable solutions, involving multiple chemical and pharmaceutical factories in several countries. This complex production chain might be jeopardised quickly in a moving geopolitical environment. Because of industrial processes protection, the antimicrobials and antidotes production chains and their localisations remain undisclosed. Nevertheless, several recent shortages are demonstrating that the supply of these medicines is at stake in Europe. To assess this risk for CBRN medical defence, we have gathered and analysed open-source medical intelligence on two keystone drugs of bio-risk medical counter-measures: Doxycycline and Ciprofloxacin.

Method: Using open source data from the database of Certificates of Suitability (CEPs) granted by the European Directorate for the Quality of Medicines and Healthcare (EDQM), crossed with pharmaceutical suppliers web-portals, the production factories of Doxycycline and Ciprofloxacin for Europe were identified.

Results: There are 17 factories worldwide having held or holding a CEP for the production of Doxycycline: 82% in China, 18% in India. There are 27 factories having held or holding a CEP for the production of Ciprofloxacin: 59% in India, 19% in China, 14% in Europe (Germany and Spain) and 7% in Mexico.

Discussion: Waiting for a complete mapping of antimicrobials and antidotes production chains and a comprehensive risk assessment of supply shortage, we can already conclude that in case of a sudden modification of the geopolitical environment impacting China, India or the supply routes linking these countries to the rest of the world, a fast and major CBRN medical counter-measures shortage is to be expected, jeopardizing CBRN defence in Europe. Short-term mitigation of this risk would be stockpilling APIs in Europe. Nevertheless, it is a very expensive policy, difficult to sustain. Long-term risk control could be the development of 3D-printing of APIs and/or a re-settlement of chemical and pharmaceutical industries able and willing to synthesise these APIs within Europe, with the support of the European Union or some of its member states.

B5

Safe containments, tailored to the application - up-scaling, modification, integration, combination and complete enclosures

Ralf Wörl[1], Thomas Hinrichs[2], and Michael Klein[3]
1- Berner International GmbH, Productmanagement, Elmshorn, Germany; 2- Berner International GmbH, General Management, Elmshorn, Germany; 3- Berner International GmbH, Head of R&D, Elmshorn, Germany

Safety cabinets are widely used technical systems to ensure personal and product protection, as well as environmental safety. Construction, design and function follow worldwide similar requirements given by standards like the European EN 12469, the American NSF/ANSI 49 or the German DIN 12980. In general, protection functions of safety cabinets are tested on the basis of these recommendations. The standard test procedures, however, do not consider in detail the later field of use and the different applications, carried out inside the containment. For example, disturbing effects that large equipment inside a safety cabinet can possess on the airflow patterns, are only taken insufficiently into account, leading to a potentially unsafe situation.

A solution to this unsatisfactory situation is the adaptation of the containment to the most challenging application combined with a coordinated testing procedure to ensure all required safety functions. The presentation gives a short overview how safety cabinets could be customised successfully to different applications. We demonstrate the up-scaling and modification of safety cabinets to offer adequate room for large equipment. Examples are given where complex devices are even more favourable integrated into the work plate level or combined laterally with the safety cabinet.

Finally, a project with the German Friedrich-

Loeffler-Institute is described, where the enclosure has been built around a device for experiments in a biosafety level 3/3+ environment. The fully customised biological safety cabinet class I was constructed for the complete integration of an inhalation exposure system for aerosol infection of laboratory animals with a lift and tilt function for the system, the installation of an incinerator and a crane system for ergonomic loading and unloading of animal cages.

Audimax / 13:30 ... 15:30

C Information for action - Surveillance and epidemiology

Chairs: Kai Kehe (DEU) and Enrico Georgi (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
C1 🖵	**Robert von Tersch** Aberdeen Proving Ground, MD, USA	US Army Public Health Center Laboratory Pilot Waste Water Surveillance for SARS-CoV-2	20+5 min
C2 🖵	**Rudolf Markt** Innsbruck, AUT	Concept for a wastewater-based SARS-CoV-2 monitoring in the multinational UN Military Camp Castor Gao (Mali)	12+3 min
C3 🧍	**Carsten Tiemann** Bad Salzuflen, DEU	Lessons learned: Large SARS-CoV-2 "Tönnies" outbreak with 1800 positive cases in a slaughterhouse - military support, laboratory challenge and sequencing results (NGS)	15+5 min
C4 🧍	**Markus Antwerpen** Munich, DEU	Innovation in the crisis: Outbreak detection of SARS-CoV-2 - Molecular diagnostics and epidemiology as a team	12+3 min
C5 🧍	**Dimitrios Frangoulidis** Munich, DEU	How electronic situation maps (SITMAP) and Dashboards can help to fight information gaps during disaster relief as in the SARS-CoV-2-pandemic	12+3 min
C6 🖵	**Erny Niederberger** Horw, CHE	Selective threat detection of airborne biological particles in real time by new AI based monitoring systems from Swisens	12+3 min
C7 🧍	**Akinyemi M. Fasemore** Munich, DEU	CoxBase: an online platform for epidemiological surveillance, visualization, analysis and typing of *Coxiella burnetii* genomic sequences	12+3 min

C1

US Army Public Health Center Laboratory Pilot Waste Water Surveillance for SARS-CoV-2

Robert von Tersch, Subrahmanyam Yerramilli, William Kowallis, and Anthony Gutierrez
US Army Public Health Center, Aberdeen Proving Ground, MD, USA

SARS-CoV-2/COVID-19 quickly transitioned from a regional outbreak to a global pandemic before science, medicine, and governments could react. Highly infectious diseases including COVID-19, potentially weaponized microbes/viruses, and antimicrobial resistant pathogens are all examples of biological threats that can quickly disrupt military mission capability. There is an urgent need for public health monitoring approaches in broader military communities that can complement individual diagnostic testing, particularly when large-scale individual testing becomes logistically cumbersome and medical treatment facilities are overwhelmed. Testing of wastewater offers a noninvasive means to detect the presence of these threats in a population at the onset of an outbreak without direct patient contact or individual testing. This approach would be of particular value in close-quarters populations such as those at military installations and facilities that are at an elevated risk for rapid spread of contagion. Recent literature suggests that the SARS-CoV-2 virus can be detected in high titers in wastewater due to fecal shedding from individuals prior to them presenting clinical symptoms and from asymptomatic carriers, including already vaccinated individuals. Thus, quantitative monitoring of this pathogen in wastewater can serve as an early warning indicator on the circulation of this virus as well as detect re-emergence of infection and spread before symptomatic cases appear. Mitigation strategies and treatment plans can then be arranged promptly to reduce impact and spread within the population to help avoid mission disruptions. Wastewater surveillance using sensitive molecular approaches offers a highly effective means to test for the presence and to quantify SARS-CoV-

2 and other pathogens shed in human waste. We have developed a high throughput capability that is precise and highly sensitive to detect the virus in wastewater samples collected prior to entering wastewater treatment plants at Army installations. This method is applicable to current and future emergent diseases which may be found in human wastewater by using quantitative polymerase chain reaction (qPCR) and may be adapted to detect other pathogenic microbes/viruses as well as to test other matrices which may be biological exposure hazards to warfighters. Finally, this study will project the resources needed to implement a sustained capability across facilities and installations.

C2

Concept for a wastewater-based SARS-CoV-2 monitoring in the multinational UN Military Camp Castor Gao (Mali)

Rudolf Markt[1], Andreas O. Wagner[1], Malena Bestehorn[2], Markus Antwerpen[2], Maximilian Klepsch[2], Reinhard Pregartbauer[3], Heribert Insam[1], Dimitrios Frangoulidis[4], and Daniela Beinkofer[4]
1- Universität Innsbruck, Microbiology, Innsbruck, Austria; 2- Bundeswehr Institute of Microbiology, Munich, Germany; 3- Wastewater solutions GmbH, Bachmanning, Austria; 4- Bundeswehr Medical Service Headquarters VI-2, Medical Intelligence and Information (MI2), Munich, Germany

The implementation and maintenance of diagnostic capabilities for SARS-CoV-2 detection in Military Missions abroad, is demanding. Periodic unfounded testing do not completely solve the problem of unidentified cases and is - beside others - a personal burden. A resource-saving and fast approach to handle this problem can be wastewater-based epidemiology. Thereby, shed genetic material of the virus in stool, collected within the catchment area of a sewage system, is analyzed to get information on the pandemic situation. Further, variant detection via whole genome sequencing (WGS) from such samples would give detailed information on the local virus-variant composition. However, easily and quickly built infrastructure for sampling, concentration of wastewater, extraction of viral RNA, and molecular-biological detection of SARS-CoV-2 needs to be established. Within the framework of a civil-military cooperation, we present here a concept for SARS-CoV-2 detection by wastewater-based epidemiology including sampling and processing of sewage water of the military UN Camp Castor near Gao(Mali) to support on-site individual diagnostic testing. Using automatic sampling devices, three fixed and three variable sampling points for upstream-tracing are used for a daily monitoring and hot-spot detection. After sampling, wastewater is processed daily in a protected setting of a 20-feet-container laboratory. Thereby, samples are concentrated via polyethylene glycol precipitation, genetic material is isolated with an automatic extraction device, and SARS-CoV-2 is detected via multiplex digital PCR including an internal process control. From PCR positive samples, RNA extracts, stabilized with a protective reagent, were sent via army postal service to appropriate equipped laboratories in Europe for additional SARS-CoV-2-WGS. Results were integrated in a comprehensive situation management, using a geographic information system (GIS) based dashboard, including all relevant aspects of a best practice COVID-19 management. The concept presented here combines a fast, continuous on-site monitoring of the SARS-CoV-2 epidemiological situation in a military camp with the option for timely further WGS based on home-country laboratory support. Therefore, wastewater-based epidemiology and surveillance is a promising tool to support individual testing strategies in the fight against the present SARS-CoV-2, emerging variants and also possible other viral caused pandemics in the future.

C3

Lessons learned: Large SARS-CoV-2 "Tönnies" outbreak with 1800 positive cases in a slaughterhouse - military support, laboratory challenge and sequencing results (NGS)

Carsten Tiemann
MVZ Labor Krone, Molec. Diagn., Bad Salzuflen, Germany

In June 2020 a large outbreak of SARS CoV-2 in our region became apparent. After screening of 250 contract workers of eastern Europe nationality at day 1, of which 221 were positive for CoV-2, an extensive screening was initiated by the health care authorities. 1800 individuals were identified positive for SARS-CoV-2 within the two following weeks. A comprehensive examination of the people living in Gütersloh was initiated. Using stationary and mobile sample collection strategies thousands of residents were tested by PCR. The main challenges were first to organize a complex screening procedure, second the timely delivery of laboratory results and finally a reliable information of the outbreak process. Due to the fact, that the health care authorities were surprised at this situation and resources for screening and evaluation tasks had to be expanded, a strong collaboration with the laboratories and most notably the support of military units of the Bundeswehr were an important requirement for the project. A harmonization of sample barcoding,

standardized collection material and a valid logistic allowed the laboratory to deliver the PCR result within few hours. The tracking team, were thus enabled to trace contact persons efficiently. Retrospectively, the courageous and purposive action of all involved parties prevented a large breakthrough into the normal population. To investigate the background of this outbreak and to identify the source of the event a study group started in August 2020 to analyze a total of 2400 positive samples by massive parallel sequencing (MPS). Since beginning of the outbreak all samples had been archived and were available for further investigation. The project ended in February 2021 with a phylogenetic analysis of 1800 positive cases of contract workers and 400 positive cases of the normal population. After finalization of this evaluation the sequence data were attached to the available metadata of the samples (age, place of residence, time of sample collection, working place, etc.). The deep analysis of this big data is still ongoing, but preliminary results are already available. The presentation should give an overview of the processes and line of actions of a sudden large outbreak, effective solutions and finally, lessons learned from this incredible scenario. The scientific postprocessing is an interesting add-on to understand the background of pathogen transmission and implementation of prevention strategies.

C4

Innovation in the crisis: Outbreak detection of SARS-CoV-2 - Molecular diagnostics and epidemiology as a team

Markus Antwerpen[1], Dimitrios Frangoulidis[2], Mathias Walter[1], Alexandra Rehn[1], A Broda[3], Sabine Zange[1], N Heyse[3], Enrico Georgi[1], E Philipp[3], Katalyn Rossmann[2], and Roman Woelfel[1]
1- Bundeswehr, Institute of Microbiology, Munich, Germany; 2- Bundeswehr, Medical Command, Medical Intelligence & Information (MI2), Munich, Germany; 3- EUTM-Mali, Gao, Mali

Since the beginning of 2020, new challenges are constantly arising for the medical service with regard to combating the spread of SARS-CoV-2. With its missions abroad, it is also important to take a special look beyond the German infection scene: New virus variants are to be detected as early as possible, evaluated and infections with these as well as introductions during repatriation to Germany are to be avoided.

With the beginning of the pandemic, InstMikroBioBw is therefore trying to develop not only an epidemiological, but also a molecular biological picture of the situation and make it available to the leadership. Since the data density was initially low, especially for the operational areas, this proved to be very difficult in some cases. However, this was successfully worked out on a continuous basis.

In mid-January 2021, an outbreak occurred among German soldiers in Mali. The now routinely established sequencing capabilities of the InstMikrobioBws made a valuable contribution, with the institute's own bioinformatic analyses providing clues in the context of infection chain elucidation. In addition, it was also possible to estimate hazard potentials for the variants prevalent there. These results opened up the possibility for the Medical Command to outline and evaluate a more concrete situation picture within the framework of its epidemiological background analyses and to initiate further analyses.

This example shows once more the necessity of a good interdisciplinary collaboration between classical epidemiology and state-of-the-art molecular biology for the sustainable well-being and safety of our soldiers in action.

C5

How electronic situation maps (SITMAP) and Dashboards can help to fight information gaps during disaster relief as in the SARS-CoV-2-pandemic

Anton Polz, Frank Fels, Gerd Großmann, Katalyn Roßmann, and Dimitrios Frangoulidis
Kdo SanDstBw, VI-2, MI2, Munich, Germany

For every military personnel it's obvious that command posts with their defined structure and situation maps belong to every tactical or strategic operation, enabling and improving decision making.

But does this works in a disaster situation where civilian personnel are in the lead?

Looking at the latest disaster situation in Germany, the COVID-19 pandemic, it turned out that many civilian leaders like heads of local governments/municipals or heads of local health offices had no proper information about the situation within their county or district.

A lot of civilian organisations like health offices, disaster control, public relation, regulatory offices, fire fighters, Technisches Hilfswerk (technical aid organisation) and police are involved in fighting against the spread of the SARS-CoV-2 disease but only the last three units are used to work with more or less paper based command posts and situation maps.

Health service support (HSS), guided by the Bundeswehr Medical command, has developed electronic dashboards and situation maps for internal and ex-

ternal use, based on ArcGIS products of the esri company, which are widely used within the Bundeswehr and increasingly in civilian governmental authorities. These dashboards show up the most important information and values and are able to show relevant values in a timeline.

The biggest advantage of such electronic dashboards is a continuous import of relevant data from different sources using (semi-) automatic working tools, reducing hands-on-time.

Relevant data can be a widespread set of different sources like hospitals, health organisations, vaccination posts, governmental units, state organisations, laboratories, NGO, military units or organizations like WHO or RKI (German National Institute of Health).

In addition, all relevant data can be spread across authorized personnel independent of the used device like PC, tablet or smart phone. Applications can be added to provide seamless order handling, tracking of support units or rare supplies too.

Our department of the Bundeswehr Medical Command, demonstrated in several assistance requests from civilian authorities, that development and usage of structured electronic dashboards did help, close information gaps and improve and speed up decision making.

C6

Selective threat detection of airborne biological particles in real time by new AI based monitoring systems from Swisens

Simone Lionetti[1], Elias Graf[2], Pascal Wullschleger[1], Yanick Zeder[2], and Erny Niederberger[2]
1- Lucerne University of Applied Sciences and Arts, Informatics, Rotkreuz, Switzerland; 2- Swisens AG, Horw, Switzerland

Bioterrorism is a latent danger because it can have a great psychological impact on the population or cause great damage to agriculture. It is necessary to arm ourselves against the new possibilities of cyberbiosecurity, which will produce new biological warfare agents. This will be possible with AI-based real-time bioaerosol identification systems that can selectively detect threats in the continuously present aerosol particle microcosm. These systems improve identification quality and reduce costly false alarms.

New-technology instruments in combination with AI-based algorithms have the potential to detect allergens and pathogens such as pollen, fungal spores and bacteria. SwisensPoleno Jupiter is a novel real-time air flow cytometer which can identify airborne particles based on Gabor holography, autofluorescence spectra and lifetime measurements. SwisensPoleno is successfully used for classification of pollen particles with sizes 10 to 100 µm thanks to deep-learning algorithms applied to holographic images (Sauvageat et al. 2020). To achieve good results for smaller particles, however, the information from fluorescence measurements is essential. In this contribution, we report on the first systematic attempt to classify particles with SwisensPoleno using relative fluorescence spectra. We demonstrate that relative fluorescence spectral data improves the performance of SwisensPoleno by discriminating between uncertain classifications when holography works well, and enables the analysis for spores around 2-3 µm where, due to the limited holographic resolution, the shape differences are not sufficient.

Preliminary results shown an overall correct identification based on relative fluorescence spectra of 84% of the following species: *A. alternata*, *Aspergillus versicolor*, *Botrytis cinerea*, *C. cladosporioides*, *C. herbarum*, *Penicillium commune*, *Stemphylium vesicarium*, *Trichoderma longibrachiatum*. These show that important species can be well distinguished. The latest results which will combine holography and fluorescence measurement data will be presented. These confirm crucial advantages of SwisensPoleno for monitoring of pathogens. Especially since Swisens provides a framework to easily extend the detection algorithms by the user.

Reference: Sauvageat, E., Zeder, Y., Auderset, K., Calpini, B., Clot, B., Crouzy, B., Vasilatou, K. (2020). Real-time pollen monitoring using digital holography. Atmospheric Measurement Techniques,13 (3), 1539-1550.

C7

CoxBase: an online platform for epidemiological surveillance, visualization, analysis and typing of *Coxiella burnetii* genomic sequences

Akinyemi M. Fasemore[1], Andrea Helbich[1], Mathias C. Walter[1], Thomas Dandekar[2], Gilles Vergnaud[3], Konrad Förstner[4], and Dimitrios Frangoulidis[5]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- University of Wuerzburg, Bioinformatics, Wuerzburg, Germany; 3- Institute for Integrative Biology of the Cell (I2BC), Paris, France; 4- ZB MED Information center for life sciences, Data Science and Services, Cologne, Germany; 5- Bundeswehr Medical Service Headquarters VI-2 Medical Intelligence & Information (MI2), Munich, Germany

Q (query) fever is an infectious zoonotic disease caused by the Gram-negative bacteria *Coxiella bur-*

netii. Although the disease has been studied since decades, it still represents a threat due to sporadic outbreaks across farms in Europe. The absence of a central platform for *Coxiella* typing data management is an important epidemiological gap which is relevant in the case of an outbreak. To fill this gap, we have designed and implemented an online, open-source, and, web-based platform called CoxBase (https://coxbase.q-gaps.de). This platform includes a database that holds genotyping information of more than 400 *Coxiella* isolates alongside metadata that annotates them. We have also implemented features for *in silico* genotyping of completely or minimally assembled *Coxiella* sequences using five different typing methods, querying existing isolates, visualization of isolate's geodata via aggregation on a world map and submission of new isolates. We tested our *in silico* typing method on 50 *Coxiella* genomes downloaded from the RefSeq database and we successfully genotyped all except for cases where the sequence quality was poor. We identified new spacer sequences using our implementation of the MST *in silico* typing method, and established *adaA* gene phenotypes for all 50 genomes as well as their plasmid types.

Garden Hall / 13:30 ... 15:30

D

Four- and eight-legged warriors

Chairs: Gerhard Dobler (DEU) and Jens P. Teifke (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
D1 🖵	**Gulnar Omasheva** Almaty, KAZ	Circulation of arthropod-borne infections in Kazakhstan	12+3 min
D2		[WITHDRAWN]	
D3 🧍	**Simone Eckstein** Munich, DEU	Prevalence of bacterial and viral zoonoses in dromedary camels from southern Tunisia	12+3 min
D4 🖵	**Saskia Schmitz** Hannover, DEU	Seroprevalence and risk factors for hantavirus-disease and leptospirosis in a hantavirus high-risk region in Lower Saxony, 2019	12+3 min
D5 🧍	**Edith Wagner** Munich, DEU	Prevalence of Puumala and Tula orthohantaviruses in *Clethrionomys glareolus* and *Microtus* spp. in Thuringia, Germany	12+3 min
D6 🧍	**Florian Lienert** Zug, CHE	The changing epidemiology of human monkeypox – a potential threat? A systematic review	12+3 min

D1
Circulation of arthropod-borne infections in Kazakhstan

Gulnar Omasheva[1] and Alim Aikimbayev[2]
1- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Reference laboratory for the control of highly dangerous infections, Almaty, Kazakhstan; 2- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Scientific consultant, Almaty, Kazakhstan

A study was carried out in 2019 to monitor circulation of the transmitting vectors in the environment. The study included the ticks collected from the southern regions of Kazakhstan: Turkistan, Zhambyl, Kyzylorda oblasts.

The results of the study showed that the tularemia isolates were obtained in the areas where no such isolates had been recovered before, and they had been detected by serological tests only. Some infections such as Q fever, South African gall sickness, listeriosis were detected in the areas where they never been reported before. Active circulation of TBE was found in Zhambyl oblast which had never been endemic with regard to this infection.

Suspensions of ticks collected from Zhambyl, Kyzylorda, and Turkistan oblasts were tested bacteriologically against tularemia, 5 isolates were obtained (3,1%). The identification results showed the following: 1 isolate recovered from suspension of ticks collected from Zhambyl oblast was classified as Francisella tularensis holarctica biovar I from *D. Niveus* pool, the remaining 4 isolates recovered from suspension of ticks collected from Kyzylorda oblast were classified as Francisella tularensis holarctica biovar II from *D. Niveus* pool.

The tick suspensions from Turkistan oblast and Shymkent were tested by PCR against Crimean-Congo Hemorrhagic Fever, where 5 positive samples were detected among 64 samples (7,8%), including 2 positive samples in the tick samples *H. anatolicum*, 2 samples *R. turanicus* and 1 sample *H. a.asiaticum*.

The tick suspensions from Zhambyl and Kyzylorda oblasts were tested by ELISA against Q fever, 4 samples out of 41 samples from Zhambyl oblast tested positive for Q fever (9,7%) from pools *D. niveus*; antigen was detected in 2 samples in the samples from Kyzylorda oblast (4%) from pool *D. niveus*.

Ticks delivered from Zhambyl and Kyzylorda oblasts were PCR-tested against TBE, Lyme disease, human granulocytic anaplasmosis, human monocytic ehrlichiosis. TBE RNA was detected in 6 samples out of 41 samples from Zhambyl oblast (14,6%), 2 (4,9%) samples were positive for gall sickness and 2 samples for listeriosis (4,9%). Out of 50 samples from Kyzylorda oblast, 2 samples tested positive for TBE (RNA) (4%), and DNA of monocytic ehrlichiosis were detected in 4 samples (8%).

Thus, we see an active circulation of arthropod-borne infections in the southern regions which requires deeper genetic research.

D2

[Contribution withdrawn]

D3

Prevalence of bacterial and viral zoonoses in dromedary camels from southern Tunisia

Simone Eckstein[1], Rosina Ehmann[1], Abderraouf Gritli[2], Houcine Ben Yahia[2], Manuel Diehl[1], Roman Wölfel[1], Mohamed Ben Rhaiem[2], Susann Handrick[1], Mohamed Ben Moussa[3], and Kilian Stoecker[1]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- Ministry of National Defense, General Directorate of Military Health, Veterinary Service, Tunis, Tunisia; 3- Military Hospital of Instruction of Tunis, Department of Virology, Tunis, Tunisia

Since 2016, the Bundeswehr Institute of Microbiology (IMB) conducts the Security Cooperation for Biological Threats in Tunisia as part of the German government's Enable & Enhance initiative.

One constant threat to public health is the spread of zoonotic diseases. Therefore, active surveillance and epidemiological monitoring of pathogen circulation is essential for taking appropriate countermeasures. However, due to limited access to medical care combined with severe underreporting of cases and the lack of epidemiologically valid data, Africa carries a high burden of neglected zoonotic diseases.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic pathogen endemic in the Arabian Peninsula and neighboring countries. The virus is mainly transmitted to humans by dromedary camels and can lead to a life-threatening outbreak of disease. Therefore, free-roaming camels, especially those crossing national borders, pose a high risk for spreading MERS-CoV.

In January 2020, a German-Tunisian team collected serum samples and nasal swabs of 500 dromedary camels in southern Tunisia to investigate the natural occurrence of MERS-CoV.

We found a high ratio of dromedaries to be seropositive (80.4 %) or actively infected (19.8 %), indicating extensive MERS-CoV circulation in Northern Africa.

As the majority of the Tunisian dromedaries sampled in this study live in their natural habitat, artificial effects like crowding do not bias the results. Since this is a rare set of samples, we extended our serological analyses and screened the 500 dromedary sera for the prevalence of other viral or bacterial pathogens such as Rift Valley fever virus (RVFV) and Foot-and-Mouth disease virus (FMDV) or *Coxiella burnetii* and *Brucella* spp., respectively.

With 73.4 % seropositivity, it appears that the majority of animals has previously been exposed to the causative agent of Q-fever, *C. burnetii*. On the other hand, only 7.4 % and 1.0 % of the dromedaries had antibodies against *Brucella* spp. and RVFV, respectively. Lastly, the area seems to be free of Foot-and-Mouth Disease as none of the animals was seropositive for FMDV.

In conclusion, we found evidence of intense circulation of MERS-CoV and *C. burnetii* in southern Tunisia. Furthermore, we confirmed the presence of RVFV and *Brucella* spp. in dromedaries from this area. This knowledge not only helps to reduce the risk of infections but also to distinguish between natural origin and targeted spread in the event of a local disease outbreak.

D4

Seroprevalence and risk factors for hantavirus-disease and leptospirosis in a hantavirus high-risk region in Lower Saxony, 2019

Saskia Schmitz[1], Christina Princk[2], Kristin Meyer-Schlinkman[3], Maren Mylius[4], Sophie Rettenbacher-Riefler[1], Armin Baillot[1], Masyar Monazahian[1], and Johannes Dreesman[1]
1- Governmental Institute of Public Health of Lower Saxony, Hannover, Germany; 2- Leibniz-Institute for Prevention Research and Epidemiology, Bremen, Germany; 3- Labor Krone, Bad Salzuflen, Germany; 4- Federal Ministry of Health, Berlin, Germany

Hantavirus-disease and leptospirosis are rodent-borne zoonoses, commonly causing unspecific symptoms, with potential severe course. With no human vaccine available in Europe, protective behaviour is essential. We examined risk factors and disease knowledge in residents of a hantavirus high-risk region.

Patient sera from two general practitioners were tested for IgG antibodies against common hantavirus and *Leptospira* strains via ELISA. Questionnaires inquired potential exposures. Associations with serostatus were assessed by calculating adjusted odds ratios (aORs) via multivariable logistic regression analysis.

Of 451 participants aged 18-85 (median: 59) 232 (51.4%) were female. Seroprevalences of 11.1% (8.5-14.4) and 2.5% (1.4-4.5) were found for hantavirus and *Leptospira* antibodies respectively. 38% of

anti-hantavirus IgG positive but none of the anti-*Leptospira* IgG positive participants had been clinically diagnosed. Frequent exposure to dead rodents (>5x/ 10 years) (aOR: 3.1; 95%-CI: 1.2-8.2) or the handling of wood (2.8; 1,4-5,8) corresponded with significantly higher, living less than 100 m from water (0.3; 0.2-0.7) with significantly lower odds of positive anti-hantavirus IgG. Longer time (≥ 2hours/ week) spent in the forest (9.1; 1.9-44.0) was significantly positive, frequent cleaning of storage rooms (>5x/ 10 years) (0.3; 0.1-0.95) significantly negative associated with anti-*Leptospira* IgG. More participants were aware of hantavirus than *Leptospira* (92% vs. 14%) but only 22% knew of the disease-specific press releases of their local health authority.

We derive recommendations for the public and health authorities: to prevent hantavirus-infections keep home and work environments free of rodents and wear protective equipment like gloves and FFP3-masks when handling wood. To prevent *Leptospira*-infections clean storage rooms regularly and avoid contact to material potentially contaminated with rodent urine when in the forest. Local health authorities need to communicate health information more efficiently to the public. Increasing awareness of leptospirosis might increase the detection rate at least for symptomatic patients and improve secondary prevention.

D5

Prevalence of Puumala and Tula orthohantaviruses in *Clethrionomys glareolus* and *Microtus* spp. in Thuringia, Germany

Edith Wagner[1], Lukas Peintner[1], Kathrin Jeske[2], Stephan Drewes[2], Christian Imholt[3], Gerald Heckel[4], Christina Ehrhardt[5], Jens Jacob[3], Rainer Ulrich[2], and Sandra Essbauer[1]
1- Bundeswehr Institute of Microbiology, Department Virology and Intracelluar Agents, Munich, Germany; 2- Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany; 3- Julius Kühn-Institute (JKI), Federal Institute for Cultivated Plants, Vertebrate Research Group, Münster, Germany; 4- University of Bern, Institute of Ecology and Evolution, Bern, Switzerland; 5- Section of Experimental Virology, Institute of Medical Microbiology, Jena University - Hospital, Jena, Germany

Orthohantaviruses occur almost worldwide. In Eurasia, they may cause haemorrhagic fever with renal syndrome (HFRS) in humans. The host of Puumala orthohantavirus (PUUV) is the bank vole (*Clethrionomys glareolus*), the PUUV distribution range ends in western Thuringia. Tula orthohantavirus (TULV) resides in common voles (*Microtus arvalis*) and field voles (*M. agrestis*) across Germany.

The exact range of PUUV and patterns of TULV infections in Thuringia remain vague. *M. arvalis, M. agrestis* and *C. glareolus* were captured in Thuringia in 2019, screened by conventional RT-PCR and TULV and PUUV identified. About 13% of *Microtus* spp. TULV RNA positive and 0.55% of all *C. glareolus* were PUUV positive.

All detected TULV strains fit into the Central North clade, but cluster independently - possibly due to evolutionary processes caused by a drought in 2018.

We are now able to draw a more comprehensive picture of vole-associated orthohantavirus distribution in Thuringia. Based on these initial findings more sophisticated evolutionary investigations of the role of landscape and land use on Orthohantavirus evolution are warranted.

This study was partly funded by the Federal Environment Agency (UBA) within the Environment Research Plan of the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (JJ, grant number 3716484310) and by the Bundesministerium für Bildung und Forschung (RoBoPub consortium).

D6

The changing epidemiology of human monkeypox - a potential threat? A systematic review

Eveline M. Bunge[1], Bernard Hoet[2], Liddy Chen[3], Florian Lienert[2], Heinz Weidenthaler[4], Lorraine R. Baer[5], and Robert Steffen[6]
1- Pallas Health Research and Consultancy, Rotterdam, Netherlands; 2- Bavarian Nordic AG, Zug, Switzerland; 3- Bavarian Nordic Inc., Morrisville, USA; 4- Bavarian Nordic GmbH, Martinsried, Germany 5- Baer PharMed Consulting Ltd, Skokie, USA; 6- University of Zurich, Epidemiology, Biostatistics and Prevention Institute, Zürich, Switzerland

Monkeypox, a zoonotic disease caused by an orthopoxvirus, results in a smallpox-like disease in humans. Since monkeypox in humans was initially diagnosed in 1970 in the Democratic Republic of the Congo (DRC), it has spread to other regions of Africa (primarily West and Central), and cases outside Africa have emerged in recent years. We conducted a systematic review of peer-reviewed and grey literature on how monkeypox epidemiology has evolved, with particular emphasis on the number of confirmed, probable, and/or possible cases, age at presentation, mortality, and geographical spread.

We identified 48 peer-reviewed articles and 18 grey literature sources for data extraction. The number of human monkeypox cases has been on the rise since the 1970s, with the most dramatic increases occurring in the DRC with over 18000 suspected cases in the 2010s. The median age at presentation has increased from 4 (1970s) to 21 years (2010s). There was an overall case fatality rate of 8.7%, with significant differences between clades - Central African 10.6% (95% CI: 8.4%-13.3%) vs. West African 3.6% (95% CI: 1.7%-6.8%). Since 2003, import- and travel-related spread outside of Africa has occasionally resulted in outbreaks. Interactions/activities with infected animals or individuals are risk behaviors associated with acquiring monkeypox. Our review shows an escalation of monkeypox cases, a spread to other countries, and a growing median age from young children to young adults. These findings may be related to the cessation of smallpox vaccination, which provided some cross-protection against monkeypox, leading to increased susceptibility. The appearance of outbreaks even beyond Africa highlights the global relevance of the disease. Increased surveillance and detection of monkeypox cases are essential tools for understanding the continuously changing epidemiology of this resurging disease.

Audimax / 16:00 ... 18:00

E

Phylogenetics and genetic diversity

Chairs: Gregor Grass (DEU) and Wolfgang Beyer (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
E1 🧍	**Ben Krause-Kyora** Kiel, DEU	New life for old bones - A 5,000-year-old hunter-gatherer already plagued by *Yersinia pestis*	25+5 min
E2 🧍	**Gilles Vergnaud** Gif-sur-Yvette, FRA	How old is *Bacillus anthracis*?	25+5 min
E3 🧍	**Sara D. Schütz** Spiez, CHE	Phylogeny of *Francisella tularensis* in Switzerland using whole genome sequencing	12+3 min
E4 🖵	**Jörg Linde** Jena, DEU	Whole-Genome-Sequencing Based High-Resolution Genotyping of *Francisella tularensis* and *Bacillus anthracis* in Germany	12+3 min
E5 🧍	**Caroline Öhrman** Umeå, SWE	Reorganized Genomic Taxonomy of *Francisellaceae* Enables Design of Robust Environmental PCR Assays for Detection of *Francisella tularensis*	12+3 min
E6 🖵	**Ansgar Schulz** Greifswald, DEU	Whole-genome sequencing of various (neglected) arboviruses circulating in Africa using MinION nanopore technology	12+3 min

E1

New life for old bones - A 5,000-year-old hunter-gatherer already plagued by *Yersinia pestis*

Ben Krause-Kyora
Kiel University, Institute of Clinical Molecular Biology, Kiel, Germany

The plague, which caused a pandemic in the late Middle Ages, leading to an estimated 25 million deaths worldwide known as the "Black Death", is caused by the bacterium *Yersinia pestis* (*Y. pestis*), which occurs especially in rodents and can be transmitted to humans by fleas as well as from person to person. Recent studies have shown that the pathogen already infected humans much earlier, but how exactly it evolved, and when it became dangerous for humans are the subject of current scientific research. In the present study, the *Y. pestis* genome were analyzed found in the remains of a man who lived in what is today Latvia around 5000 years ago. The analyses provide insight into the very early stages of the evolution of *Y. pestis*. Contrary to what was previously assumed, the results show that the bacteria already infected people at the beginning of the Neolithic Period, but probably had only a limited potential for infection, so that they could not yet spread in epidemic proportions.

E2

How old is *Bacillus anthracis*?

Alexandr Shevtsov[1], Larissa Lukhnova[2], Uinkul Izbanova[2], Jean-philippe Vernadet[3], Marat Kuibagarov[1], Asylulan Amirgazin[1], Yerlan Ramankulov[1], and Gilles Vergnaud[3]
1- National Center for Biotechnology, Nur Sultan, Kazakhstan; 2- National Scientific Center for Especially Dangerous Infections named by Masgut Aykimbayev, Almaty, Kazakhstan; 3- Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France

Bacillus anthracis is one of the most dreaded bioterror agents for a number of reasons, including practical issues. One such issue is the difficulty to trace back the origin of an attack, as illustrated by the Amerithrax investigation. Consequently, the improvement of source-tracking capacities, often called microbial forensics, may constitute an efficient deterrent against such threats.

One part of microbial forensics, precise strain identification, depends upon the capacity to interpret the significance of genetic differences in terms of temporal and geographic origin. As such, a better understanding of the phylogeography of *B. anthracis* contributes to biodefense. Two controversial hypotheses have been proposed regarding the

geographic and temporal origins of *B. anthracis*. According to the first hypothesis, *B. anthracis* was already widespread across Africa and Eurasia during the late Paleolithic. The second hypothesis proposes that *B. anthracis* emerged from Central Africa a few thousand years before present during the Neolithic era, possibly as a consequence of human activities, including pastoralism. It then spread to Asia and Europe where new lineages emerged as a consequence of adaptation to local environment.

Our goal is to improve the description of *B. anthracis* present in Eurasia in order to help evaluate the two models. We have characterized a large collection of 154 strains isolated in Kazakhstan, Central Asia, in years 1950-2016 by a combination of genotyping and whole genome sequencing approaches. By comparing the new data with publicly available sequence data, we show that most strains from Kazakhstan define specific, closely related and previously unknown sublineages. The results allow the dating of one important node along the phylogeny of *B. anthracis*. Together with historical facts, our observations suggest that the diversification of these sublineages occurred after the collapse of the 13th century global Mongol Empire and provide support in favor of the Neolithic *B. anthracis* model.

E3

Phylogeny of *Francisella tularensis* in Switzerland using whole genome sequencing

Sara D. Schütz, Susanne Thomann, Kristina M. Schmidt, and Matthias Wittwer
Spiez Laboratory, Biology, Spiez, Switzerland

Whole genome sequencing (WGS) methods provide new possibilities in the field of molecular epidemiology. This is particularly true for monomorphic organisms where the discriminatory power of traditional methods (e.g., restriction enzyme length polymorphism typing, multi locus sequence typing etc.) is inadequate to elucidate complex disease transmission patterns, as well as resolving the phylogeny at high resolution on a micro-geographic scale. In this study, we present insights into the population structure of *Francisella tularensis* subsp. *holarctica* (*Fth*), the causative agent of tularemia, in Switzerland. In a study from Wittwer et al. of 2018, 59 *Fth* isolates were obtained from castor bean ticks (*Ixodes ricinus)*, animals and humans. Together with additional 45 *Fth* isolates collected mainly from patients during the past three years, a high-resolution phylogeny was inferred using WGS methods. The majority of the *Fth* population in Switzerland belongs to the western European B.11 clade and shows an extraordinary genetic diversity underlining the old evolutionary history of the pathogen in the alpine region. Moreover, a new B.11 subclade was identified, which was not described so far. The combined analysis of the epidemiological data of human tularemia cases together with the whole genome sequences of the 59 isolates provide evidence that ticks play a pivotal role in transmitting *Fth* to humans and other vertebrates in Switzerland. This is further underlined by the correlation of disease risk estimates with climatic and ecological factors influencing the survival of ticks.

E4

Whole-Genome-Sequencing Based High-Resolution Genotyping of *Francisella tularensis* and *Bacillus anthracis* in Germany

Jörg Linde, Mostafa Y. Abdel-Glil, Mandy Elschner, and Herbert Tomaso
Friedrich-Loeffler-Institute, Institute for Bacterial Infections and Zoonoses, Jena, Germany

Advances in sequencing technologies have paved the way for routine whole-genome sequencing of bacterial pathogens. While genome data offer a large amount of information and applications, genotyping at highest resolution is probably the most important application especially for potential agents of bioterrorism.

Here, we performed genotyping of 477 animal samples from *Francisella tularensis*[1] - the causative agent of the zoonotic disease tularemia and eight animal samples from three recent *Bacillus anthracis* outbreaks in Germany [2]. Typing was performed using both Single Nucleotide Polymorphism (SNP) based typing and core-genome multi-locus sequence typing (cgMLST), which we first established for *B. anthracis* [2].

All *F. tularensis* strains were of subsp. *holarctica* and showed the typical pattern of major clade B.6 dominating in western part and B.12 in eastern part of Germany. The subclade distribution, both on local and national levels, among strains from humans and hares was similar, suggesting circulation of the same genotypes both, in animals and humans. Whilst close to identical isolates of the same subclade were found distributed over large areas, small geographical foci often harbored members of different subclades.

For *B. anthracis* two strains from an outbreak in the Bavarian Alps 2009, five strains from an outbreak 2012 in Stendal (Saxony-Anhalt) and three strains from an outbreak 2014 in Dobichau (Saxony-Anhalt) were sequenced. While the strains from Bavarian

were clade B all strains from Saxony-Anhalt were clade A.Br.001/002 and could not be further discriminated by MLVA. Using cgMLST, a maximum of five allelic variations could be identified between isolates from each single outbreak. The two outbreaks in Saxony-Anhalt were due to strains that were distant from each other by 30 different alleles, which might indicate different sources of the outbreaks, precluding a direct transmission of strains between these outbreaks.

In conclusion, high-resolution genotyping of *F. tularensis* and *B. anthracis* from animal sources will help to identify unnatural events in Germany.

References:

1. Linde *et al.* (2020), Microorganisms, 2020 Dec 5;8(12), PMID: 33291395
2. Abdel-Glil *et al.* (2021), J Clin Microbiol, 2021 Jun 18;59(7), PMID: 33827898

E5

Reorganized Genomic Taxonomy of *Francisellaceae* Enables Design of Robust Environmental PCR Assays for Detection of *Francisella tularensis*

Caroline Öhrman[1], Jason Sahl[2], Andreas Sjödin[1], Ingrid Uneklint[1], Rebecca Ballard[2], Linda Karlsson[1], Ryelan F. Mcdonough[2], David Sundell[1], Kathleen Soria[2], Stina Bäckman[1], Kitty Chase[3], Björn Brindefalk[1], Shanmuga Sozhamannan[4], Adriana Vallesi[5], Emil Hägglund[1], Jose gustavo Ramirez-paredes[6], Johanna Thelaus[1], Duncan Colquhoun[7], Kerstin Myrtennäs[1], Dawn Birdsell[2], Anders Johansson[8], David M. Wagner[2], and Mats Forsman[1]
1- Swedish Defence Research Agency (FOI), CBRN Defence and Security, Umeå, Sweden; 2- Northern Arizona University, Pathogen and Microbiome Institute, Flagstaff, USA; 3- Fort Detrick, US Army Medical Research Institute, Frederick, USA; 4- CBRND Enabling Biotechnologies (JPL CBRND EB), Logistics Management Institute supporting Defense Biological Product Assurance Office (DBPAO) Joint Project Lead, Frederick, USA; 5- University of Camerino, School of Biosciences and Veterinary Medicine, Camerino, Italy; 6- Ridgeway Biologicals Limited a Ceva Santé Animale Company, Compton, United Kingdom; 7- Norwegian Veterinary Institute, Fish Health Research Group, Oslo, Norway; 8- Umeå University, Department of Clinical Microbiology, Clinical Bacteriology, and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå, Sweden

Discrimination of pathogenic *Francisella tularensis* from closely related non-harmful variants frequently found in environmental samples has historically been a challenge. In the US BioWatch program which conducts surveillance of pathogens in air samples a majority of the 149 positive results between 2003 and 2014 were associated with assays for *F. tularensis*. Given the ubiquity of diverse *Francisella* species in the environment, and the fact that many of them can apparently become aerosolized, it seems likely that the presence of near-neighbours to *F. tularensis* on air filters are the source of these false positive BioWatch results.

We have sequenced new isolates of the family *Francisellaceace* and together with public data collected a comprehensive set of genome sequences representing not only the human clinically relevant species *F. tularensis,* causing tularemia, but also its nearest neighbors. We have reorganized the genomic taxonomy of *Francicellaceace* with support of the Genomic Taxonomy Database (https://gtdb.ecogenomic.org/) and described the current complete phylogenetic structure.

In the reorganized taxonomy *Francisella* belongs to one of three major branches, here defined as the "*Francisella* near neighbor" (FNN) dataset, including 327 *F. tularensis* genomes and 172 non-*F. tularensis* genomes. The genus *Francisella* have four main branches and *F. tularensis* is one of 11 species within clade 1. After extracting unique regions amongst all genomes we found 6 regions unique for *F. tularensis*. We performed both in silico and wet-bench validation against a panel of diverse strains and PCR assay Ft-sp.FTS_0772 of region 1 demonstrated superior or equivalent specificity compared to previously published PCR assay.

This study dramatically expands the genome space of *Francisellaceae*. Previous diagnostics for *F. tularensis* have suffered from a largely shared but unexplored *Francisellaceae* pan genome, resulting in false positive results, which is problematic for routine biothreat surveillance. The unique signatures identified in this study may also be shared by unexplored, unculturable, and previously undetected *Francisella* near neighbor species that have yet to be characterized. Continued culture-independent characterization of these cryptic species will expand our understanding of the diversity of these lineages.

E6

Whole-genome sequencing of various (neglected) arboviruses circulating in Africa using MinION nanopore technology

Ansgar Schulz[1], Balal Sadeghi[1], Franziska Stoek[1], Jacqueline King[2], Martin Eiden[1], and Martin H. Groschup[1]
1- Friedrich-Loeffler-Institut, INNT, Greifswald,

Germany; 2- Friedrich-Loefflер-Institut, IVD, Greifswald, Germany

The majority of emerging infectious diseases are caused by zoonotic pathogens, with one in four pathogens being of viral origin. Among those, the importance of vector-borne diseases has greatly increased within the last decades. Especially in Africa, there are a large number of (neglected) arthropod-borne viruses (arboviruses) with a zoonotic potential, and yet little is known of most of these pathogens, including their actual distribution or genetic diversity. In addition to reliable molecular diagnostics, such as RT-qPCR, the generation of longer gene sequences up to the complete genome of the detected pathogens using next-generation sequencing (NGS) techniques has therefore become the focus of interest. For this purpose, we used the portable MinION instrument (Oxford Nanopore Technologies, Oxford, United Kingdom) to sequence six different arboviruses that are known to be circulating in Africa including Crimean-Congo hemorrhagic fever virus (CCHFV) and Rift Valley fever virus (RVFV). Especially the compact dimensions of the device are a great advantage, which allows easier procurement even for laboratories with limited access to expensive high-end laboratory equipment. We evaluated the effectiveness of the non-specific viral quantification technique SISPA (Sequence-Independent Single-Primer-Amplification) by comparing the sequencing results of the different viruses with each other. The promising results of this study contribute to providing viral sequences of neglected tropical arboviruses, allowing a better understanding of their genetic diversity and distribution.

Garden Hall / 16:00 ... 18:00

F

Case reports and educational session

Chairs: Sabine Zange (DEU) and Dieter Hoffmann (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
F1 🖵	**Susann Dupke** Berlin, DEU	Heatwave associated *Vibrio* infections in Germany 2018 and 2019	20+5 min
F2 🖵	**Christiane P. Tiefenbacher** Wesel, DEU	Surprising diagnosis in a patient with pericardial effusion	15+5 min
F3 🧍	**Frank Sitterlee** Neubrandenburg, DEU	The Hunter and the Hip	15+5 min
F4 🧍	**Johannes P. Borde** Oberkirch, DEU	Three patients with diseases of different etiology after tick bite in Southern Germany	20+5 min
F5 🧍	**Sonja Mantel** Munich, DEU	A case of community-aquired pneumonia in a security guard in times of Corona	15+5 min

F1

Heatwave associated *Vibrio* infections in Germany 2018 and 2019

Susann Dupke[1], Thomas T. Brehm[2], Laura Berneking[3], Meike S. Martins[4], Daniela Jacob[1], Holger Scholz[1], Oliver Drechsel[5], Karsten Becker[6], Axel Kramer[7], Martin Christner[8], Martin Aepfelbacher[8], Stefan Schmiedel[2], and Holger Rohde[8]

1- Robert Koch-Institute, ZBS 2: Centre for Biological Threats and Special Pathogens, Highly Pathogenic Microorganisms, Berlin, Germany; 2- University Medical Center Hamburg-Eppendorf, I. Department of Internal Medicine, Hamburg, Germany; 3- University Medical Center Hamburg-Eppendorf, Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany; 4- Institut für Meereskunde, Centrum für Erdsystemwissenschaften und Nachhaltigkeit, University Hamburg, Hamburg, Germany; 5- Robert Koch-Institute, MF1: Bioinformatics, Berlin, Germany; 6- Friedrich Loeffler-Institute of Medical Microbiology, University Medicine Greifswald, Greifswald, Germany; 7- Institute of Hygiene and Environmental Medicine, University Medicine Greifswald, Greifswald, Germany; 8- German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, Hamburg, Germany

Vibrio spp. are aquatic bacteria that preferentially grow in warm seawater with moderate salinity. Beside the O:1 and O:139 serovar of *V. cholerae*, the causative agent of cholera, several *Vibrio* species have been recognized as human pathogens causing wound infections, gastroenteritis and ear infections. During the summer months of 2018 and 2019, unprecedented high sea surface temperatures were recorded in the German Baltic Sea, leading to a large number of human *Vibrio* infections with different manifestations. We performed an observational retrospective multi-center cohort study of patients diagnosed with domestically acquired *Vibrio* infections in Germany in 2018 and 2019. Demographic, clinical, and microbiological data were assessed. Available isolates were subjected to whole-genome sequencing and antimicrobial susceptibility testing. We identified 63 patients with *Vibrio* infections, the majority of which were contracted between June and September, primarily in the Baltic Sea. 38 patients (60%) presented with wound infections, 16 (25%) with ear infections, six (10%) with gastroenteritis, two (3%) with primary septicemia, and one (2%) with pneumonia after seawater aspiration. The majority of infections were attributed to *V. cholerae* (non-O1/non-O139) (n=30; 48%) or *V. vulnificus* (n=22; 38%). Phylogenetic analyses showed clusters of identical strains of *V. vulnificus* that caused wound infections in different geographic locations, suggesting that certain clonal lines could spread across the Baltic Sea. This study clearly shows that severe heatwaves during the summer months of 2018 and 2019 have facilitated a large number

of *Vibrio* infections contracted in Germany. Since the ongoing climate change is likely to favor the proliferation of these bacteria, a further increase in *Vibrio*-associated diseases may be expected in the future. People with previous illnesses or a weakened immune system would be particularly at risk of infection with *Vibrio* spp.

F2
Surprising diagnosis in a patient with pericardial effusion

Christiane P. Tiefenbacher[1], Moaaz Elsharabassy[1], Jan Gummert[2], and Sabine Zange[3]
1- MHWesel, Cardiology, Wesel, Germany; 2- Herzzentrum , Heart surgery, Bad Oeynhausen, Germany; 3- Bundeswehr Institute of Microbiology , Munich, Germany

No abstract due to interactive voting session.

F3
The Hunter and the Hip

Frank Sitterlee[1], David Beier[1], Sabine Zange[2], and Dirk Ganzer[1]
1- Dietrich-Bonhoeffer Klinikum Neubrandenburg, Klinik für Orthopädie und Unfallchirurgie, Neubrandenburg, Germany; 2- Bundeswehr Institute of Microbiology, Munich, Germany

No abstract due to interactive voting session.

F4
Three patients with diseases of different etiology after tick bite in Southern Germany

Johannes P. Borde[1], Gerhard Dobler[2], and Sabine Zange[2]
1- Praxis Dr. Borde, Gesundheitszentrum Oberkirch, Oberkirch, Germany; 2- Bundeswehr Institute of Microbiology, Munich, Germany

No abstract due to interactive voting session.

F5
A case of community-aquired pneumonia in a security guard in times of Corona

Sonja Mantel[1,2], Sabine Zange[1], and Stephan Opderbeck[2]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- Bundeswehrkrankenhaus Ulm, Dept of Internal Medicine, Ulm, Germany

No abstract due to interactive voting session.

Audimax / 08:30 ... 10:30

G

Last minute highlights: Aspects of zoonotic infections - from detection to therapy

Chairs: Rosina Ehmann (DEU) and Steve Lonsdale (GBR)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
G1 🧍	**Jean-Lou Marié** Tours, FRA	SARS-CoV-2 antibodies seroprevalence in dogs from France using ELISA and an automated western blotting assay	12+3 min
G2 🖵	**Esther Schalke** Ulmen, DEU	Scent dog identification of SARS-CoV-2-infections - double blind studies	12+3 min
G3 🧍	**Ulrich Schotte** Kronshagen, DEU	Selected Biological Agents from the Veterinary Perspective	12+3 min
G4 🖵	**Kevin Yeo** Marlow, GBR	Use of Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) - (Equine) (BAT®) in Clinical Study Subjects and Patients: A 15-Year Systematic Safety Review	12+3 min
G5 🖵	**Michela Puxeddu** Rome, ITA	Discovery of New SARS-CoV-2 Inhibitors	12+3 min
G6 🧍	**Isabel Hunger-Glaser** Spiez, CHE	The new WHO BioHub at the Spiez Laboratory	12+3 min
G7 🖵	**Lokesh Joshi** Galway, IRL	A nature inspired approach to pathogen binding for decontamination, sample collection and diagnostics	12+3 min
G8 🧍	**F. Nina Papavasiliou** Heidelberg, DEU	A novel immunization platform for the elicitation of potent antibodies that block opioid intoxication and prevent overdose	12+3 min

G1

SARS-CoV-2 antibodies seroprevalence in dogs from France using ELISA and an automated western blotting assay

Younes Laidouidi[1], Youssouf Sereme[1], Hacène Medkour[1], Stéphanie Watier-Grillot[2], Pierre Scandola[2], Jacques Ginesta[2], Virginie Andréo[2], Claire Labarde[2], Loïc Comtet[3], Philippe Pourquier[3], Jean-Lou Marié[2], and Bernard Davoust[1]
1- IHU Méditerranée Infection, Marseille 05, France; 2- French military health service, Tours, France; 3- Innovative Diagnostics, Grabels, France

Dogs are occasionally receptive to SARS-CoV-2. They develop few or no clinical signs.

Epidemiosurveillance of SARS-CoV-2 in dogs requires testing to distinguish it from other canine coronaviruses. Over the last year, significant progress has been made in the diagnosis of SARS-CoV-2, enabling its surveillance in humans and animals. Here, using ELISA and automated western blotting (AWB) assays, we performed a longitudinal study on 809 apparently healthy dogs from different regions of France to investigate anti-SARS-CoV-2 antibodies. There were three principal groups: (i) 356 dogs sampled once before the pandemic, (ii) 235 dogs sampled once during the pandemic, and (iii) 218 dogs, including 82 dogs sampled twice (before and during the pandemic), 125 dogs sampled twice during the pandemic and 11 dogs sampled three times (once before and twice during the pandemic). Using ELISA, the seroprevalence was significantly higher during the pandemic [4.9% (22/453)] than in the pre-pandemic period [1.1% (5/448)]. At least 8 ELISA-seroconversions were observed among the 218 dogs sampled twice. ELISA positive sera before the pandemic were not confirmed in serial testing by AWB, which suggests a possible cross-reactivity of the ELISA, probably with other canine coronaviruses. No significant difference was observed between these two serological tests (Q=1.455, p=0.228). Positive correlation was observed between the SARS-CoV-2 seroprevalence in dogs and the incidence of the infection in humans. The AWB could be used as a second line assay

to confirm the doubtful and discrepant ELISA results in dogs. Our findings confirm the previous experimental models concerning the receptivity of dogs to SARS-CoV-2. They suggest the weak or absence of the virus transmission from the infected to noninfected dogs or humans. However, the new variants with multiple mutations could adapt to dogs; this hypothesis cannot be ruled out in the absence of canine SARS-CoV-2 genomic data.

G2

Scent dog identification of SARS-CoV-2-infections - double blind studies

Esther Schalke[1] and Holger A. Volk[2]
1- SDstHundeBw, Ulmen, Germany; 2- University of Veterinary Medicine Hanover, Departement of Small Animal Medicine and Surgery, Hanover, Germany

Summary: The COVID-19 pandemic has rapidly spread across the globe. Rapid testing remains one of the main strategies to contain the spread. Scent dogs are capable of detecting disease-specific volatile organic compounds emanated from infected body cells and could support current testing strategies.

Methods/Results: In a pilot study ten dogs were trained to detect SARS-CoV-2 infections in inactivated saliva samples. They were able to discriminate between samples from SARS-CoV-2 infected patients and negative controls. The subsequent transfer performance for the recognition of non-inactivated samples and detection accuracy were tested different non-inactivated body fluids (saliva, urine, sweat) in a randomised, double-blind study. Dogs were tested on a total of 5242 randomised samples. Dogs detected non-inactivated saliva samples with an average sensitivity of 84% and specificity of 95%. In the comparison between the three body fluids, sensitivity and specificity were 95% and 98% for urine, 91% and 94% for sweat, 82%, and 96% for saliva. A third study evaluated the dogs' ability to distinguish SARS-CoV-2 from other viral infections. Nasopharyngeal swab samples and cell culture samples from 15 viruses were presented as distractors in a randomized, double-blind study. Dogs were either trained with SARS-CoV-2 positive saliva samples (scenario I) or supernatant from cell cultures (scenario (II and III). In scenario I dogs detected positive with a mean sensitivity of 73.8% and a specificity of 95.5%. In test scenario II and III cell culture supernatant from cells infected with SARS-CoV-2, cells infected with other coronaviruses and non-infected cells were presented. Dogs achieved mean sensitivities of 61.2% (scenario II) and 75.8% (scenario III). The specificities were 90.9% (scenario II) and 90.2% (scenario III).

Conclusions: Detection dogs were able to transfer the conditioned scent detection of inactivated saliva samples to non-inactivated saliva, urine and sweat samples.In the three other test scenarios the mean specificities were above 90% which indicates that dogs can distinguish SARS-CoV-2-infections from other viral infections. However, compared to earlier studies our scent dogs achieved lower diagnostic sensitivities.To deploy COVID-19 detection dogs as a reliable screening method it is therefore mandatory to include a variety of samples from different viral respiratory tract infections in dog training to ensure a successful discrimination process. Detection dogs may provide a reliable screening method for SARS-CoV-2 infections.

G3

Selected Biological Agents from the Veterinary Perspective

Ulrich Schotte, Katharina Marquart, Laurence Johanson, and Anja Petrov
ZInstSanBw Kiel, Dep A - Veterinary Medicine, Kronshagen, Germany

Most infectious diseases of concern are zoonoses serving as an ideal platform for interdisciplinary cooperation and research from the one health perspective. In the context of zoonotic infectious diseases veterinarians focus on the highly sensitive detection and identification of possible reservoirs and / or vectors for the respective pathogen. In contrast to this, the highly specific detection capability of clinical cases of foreign animal diseases of concern is regulated by the European Animal health Law. The case definition is strictly defined including diagnostic standards up to the certification of commercial diagnostic test kits, exclusively allowed for diagnostic purposes. Most importantly, licensed molecular and serological techniques in compliance with the German Animal Health Law have to be applied for laboratory diagnostics. This often requires additional test validation, when samples from medical and veterinary points of view have to be investigated.

The actual extent of the HPAI- and ASFV-outbreaks allow a tentative view in what outcome the (illegal) introduction of foreign animal diseases can have; concerning these outbreaks, the European Union as well as Germany were prepared through regularly updated risk assessments based on their natural epidemiology and characteristics. Although the HPAI-outbreak 2020/21 was the largest outbreak in Central Europe since decades, the implementation of control measures prevented the further spread within farmed poultry even in high-density areas. Nevertheless, around 2 million of birds died until the

end of June 2021 in Germany. The ASFV-outbreak in Europe shows a completely different picture. In contrast to older literature the ASF-Virus is not highly contagious, the low contagiosity complemented with its high lethality of nearly 100% in naive populations. Long-term survival in carcasses and in food from swine origin yields to a long local persistence. The diagnostic capabilities have to ensure valuable and robust results also from carcasses in various stages of decay including bones. Since ASF was introduced into Germany in 2020, the first ASF case on a military training area was detected in February 2021. Military activities in the affected areas are reduced to the absolute minimum in order to prevent a further spread of the disease. Thus the control of foreign and threatening animal diseases is also a military duty with the aim to improve the civil-military cooperation.

G4

Use of Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) - (Equine) (BAT®) in Clinical Study Subjects and Patients: A 15-Year Systematic Safety Review

Kevin Yeo[1], Geraldine S. Parrera[2], Hugo Astacio[2], Priya Tunga[3], Deborah M. Anderson[3], Christine L. Hall[3], Laura Cochrane[4], and Jason S. Richardson[3]
1- Emergent BioSolutions, Director, Global Medical Affairs, Marlow, United Kingdom; 2- Emergent BioSolutions Canada, Pharmacovigilance, Winnipeg, Canada; 3- Emergent BioSolutions Canada, Clinical Development, Winnipeg, Canada; 4- Emergent BioSolutions, Senior Director, Global Medical Affairs, Tewkesbury, United Kingdom

Introduction: Botulism is a rare, life-threatening, sometimes fatal paralytic illness caused by neurotoxins produced by *Clostridium botulinum* bacteria. BAT® [Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) - (Equine)] is an equine-derived heptavalent botulinum antitoxin indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A, B, C, D, E, F, or G in adult and pediatric patients.

Method: We calculated the adverse event (AE) incidence rate for BAT product from 2006-2020 using two denominators. The AE incidence rate was first calculated conservatively using only BAT product-exposures for individuals with a record (512 exposures) from pre and post-market studies as well as post-marketing AE data. The AE incidence rate was alternately estimated using total exposure to BAT product using pre-licensure data as well as post licensure deployment information (1128 exposures).

Results: This analysis focuses on hypersensitivity, as it is a common risk for antitoxins of equine origin. We conclude that BAT product-exposed individuals experienced hypersensitivity at a rate of 2.3-5.1% and serum sickness at a rate of 0.09-0.2%. We used the Brighton Collaboration Anaphylaxis Working Group to define the parameters of anaphylaxis. Anaphylaxis occurred in 0.62-1.37% of BAT product-exposed individuals. Overall, the most frequently reported BAT product-related AEs occurring in greater than 1% of the 512-1128 BAT-product exposed individuals were pyrexia, tachycardia, bradycardia, and blood pressure increase reported in 1.8-3.9%, 1.0-2.2%, 0.89-2.0% and 0.62-1.4% of individuals respectively. This data supports continued use of BAT product with an acceptable safety profile for patients with known or suspected botulism.

Discussion: The findings conclude that BAT product has an acceptable safety profile for subjects in controlled clinical studies and in patients that received BAT product. For patients properly managed in an intensive care setting, there is a favorable benefit-risk profile. Adverse events of special interest including bradycardia, hemodynamic instability, hypersensitivity reactions, serum sickness, and febrile reactions in the BT-010 safety registry were specifically solicited and may have contributed to an increased reporting.

G5

Discovery of New SARS-CoV-2 Inhibitors

Michela Puxeddu[1], Friederike Hucke[2], Eloise Mastrandelo[1], Mario Milani[1], Marianna Nalli[1], Giuseppe La regina[1], Joachim J. Bugert[2], and Romano Silvestri[1]
1- Sapienza University of Rome, Department of Drug Chemistry and Technologies, Rome, Italy; 2- Institut f. Mikrobiologie der Bw, Department of Virology, Munich, Germany

There is an urgent need of effective drugs for the treatment of the SARS-CoV-2 infection. Remdesivir (Veklury), a nucleotide analogue, is the only drug that was approved by FDA on 22-Oct-2020 for certain COVID-19 patients.

We performed virtual screening studies of a proprietary compound library of >6000 molecules against conserved SARS-CoV-2 enzymes, namely papain-like protease (PLpro), 3C-like proteinase (3CLpro) e RNA-dependent RNA polymerase (RdRp).

In these preliminary docking studies, we identified compounds with EC_{50} values in low micromolar range of concentration against SARS-CoV-2 mucIMB and mucIMB-CB (alpha variant) strains in Vero B6 cells. In particular, compound RS2523

inhibited the SARS-CoV-2 PLpro with IC_{50} = 27 microM and the SARS-HCoV-OC43 strain withIC_{50} = 4.3 microM.

Compound RS2523 was selected as a lead compound for optimization studies.

G6

The new WHO BioHub at the Spiez Laboratory

Isabel Hunger-Glaser
1- Spiez Laboratory, Biology, Spiez, Switzerland

The WHO BioHub System has been established in Mai 2021 and will allow Member States to share biological materials with epidemic or pandemic potential (BMEPP) on a voluntary basis. The goal of this WHO BioHub System is to encourage the sharing of BMEPP soon after the detection of a pathogen with epidemic or pandemic potential in order to characterize it, provide information and foster greater international cooperation for health emergency preparedness and response.

The first facility in this system is the Spiez Laboratory recieving in the pilot phase variants of SARS-CoV-2.

The objectives of the WHO BioHub System will be to:

- Promote rapid and timely sharing of BMEPP;
- Facilitate the rapid characterization of such pathogens and related risk assessment;
- Facilitate the development of safe, protective countermeasures and other public health products;
- Ensure fair and equitable access to such products by all countries based on public health needs.

G7

A nature inspired approach to pathogen binding for decontamination, sample collection and diagnostics

Lokesh Joshi[1], Justine O'sullivan[2], and Stephen Cunningham[1]
1- National University of Ireland Galway, Advanced Glycoscience Research Group, Galway, Ireland; 2- Aquila Bioscience Ltd, Galway, Ireland

The global health crisis and environmental crisis are converging and as a result infectious diseases are on the rise. COVID-19 pandemic is a pertinent example of this challenge. There is an urgent need to develop medical countermeasures that are not only effective but are also environmentally friendly and do not add to the damage caused by many of the current interventions and products.

Chemical disinfection has been the most common method of controlling pathogens for over a century. However, currently all disinfection and decontamination products are based on broad stroke biocides and other chemicals that have harmful effect on human skin and respiratory system. Moreover, these chemicals cause damage to the environment and decimate beneficial microorganisms.

With the funding from European Defence Agency and European Innovation Council, Aquila Bioscience and National University of Ireland Galway, are developing innovative technologies to decontaminate surfaces, including human skin and mucosal surfaces, from viral, bacterial and fungal pathogens and biological toxins in a safe and effective manner. We are also generating epitope specific binder molecules to target infectious agents of interest. This strategy has clear potential for decontamination of sensitive equipment and skin, bioagent sample collection, enrichment and forensic/diagnostic analysis.

G8

A novel immunization platform for the elicitation of potent antibodies that block opioid intoxication and prevent overdose

J. Verdi, G. Triller, E. Vlachou, J. Zeelen, M. van Straaten, M. Pravetoni, E. Stebbins, and F. Nina Papavasiliou
Deutsches Krebsforschungszentrum, Heidelberg, Germany

Many previous attempts to generate antibodies against small molecules, such as fentanyl and carfentanyl, have employed conventional antigen carrier systems that have so far proven insufficient in this space. We have developed a novel antibody elicitation platform that is characterized by extraordinary epitope focusing and does not require the addition of any adjuvants to generate strong responses. The platform is based on the uniquely repetitive and antigenic surface coat of *Trypanosoma brucei*, comprised in large part by variant surface glycoprotein (VSG). The repetitive and dense nature of this coat provides a robustly-antigenic surface, leading to an extremely epitope-focused immune response.

We have most recently applied our platform, the *VAST* (VSG-immunogen Array by Sortase Tagging), to the opioid fentanyl in hopes of designing both an overdose preventing vaccine and overdose stopping passive therapies. The platform is generated

through the sortase-mediated tagging of fentanyl (or any other "sortaggable" antigen) to VSG, followed by inactivation of the parasites prior to injection. We show that our vaccine reproducibly protects rodents from fentanyl intoxication by trapping fentanyl in the serum and thus preventing access to the brain-resident opioid receptors. We have also harvested B cells from the vaccinated animals, sequenced those cells, and quickly identified low picomolar affinity candidate antibodies to be used as passive therapeutics.

The trypanosome-based antibody elicitation platform offers a new avenue to design immunotherapeutics and diagnostics specific to "difficult" antigens like fentanyl and other small molecules that are of relevance in the counterdefence space.

Garden Hall / 08:30 ... 10:30

H

A tight match: host vs. pathogen

Chairs: Haim Levy (ISR) and Heiner von Buttlar (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
H1 🧍	**Haim Levy** Ness Ziona, ISR	The neglected virulence factor – Role of the capsule in *Bacillus anthracis* pathogenicity	20+5 min
H2 🖵	**Yasuyuki Tsunoi** Tokorozawa, JPN	Viability improvement of three-dimensional cultured skin substitutes by photobiomodulation	12+3 min
H3 🖵	**Voahangy Andrianaivoarimanana** Antananarivo, MDG	Long- term persistence of humoral immune response against plague in Malagasy patients	12+3 min
H4 🧍	**Marina Šantic** Rijeka, HRV	*Francisella tularensis*-one peculiar and possibly emerging pathogen	12+3 min
H5		[WITHDRAWN]	
H6		[WITHDRAWN]	
H7 🧍	**Gvantsa Brachveli** Tbilisi, GEO	Multiple SARS-CoV-2 Mutations in an Immunocompromised Oncology Patient	12+3 min

H1

The neglected virulence factor? Role of the capsule in *Bacillus anthracis* pathogenicity

Haim Levy
Israel Institute for Biological Research, Department of Infectious Diseases, Ness Ziona, Israel

Bacillus anthracis pathogenesis relies on the orchestrated activity of two major virulence systems; the tripartite toxins and the poly-δ-D- glutamic acid capsule. Inactivation of each of these systems abolishes the spores? ability to infect the host. While the role of the toxins was extensively studied in vitro and in vivo, little is known on the role of the capsule, other than its proposed role in survival in phagocytic cells of the immune system. Previously we have demonstrated that while the toxins are essential in the first stages of establishing an effective infection, once blood borne *B. anthracis* will kill the host even in the absence of active toxins. To study the role of toxins and capsule in the systemic phase of anthrax, we used an intravenous (IV) infection system using vegetative pre-conditioned bacteria. This system enabled the examination of the different virulence factors? roles in the deadly advanced stage of the disease.
The existence of Anthrax related meningitis has been established in humans and non-human primates, primarily by autopsy. We demonstrated a similar pathology in rabbits and Guinea pigs following infection with fully virulent strain or with a toxins null mutant. In all of these cases meningitis rather than encephalitis was observed. However, IV infection of a toxinogenic, non-encapsulated (vaccine) strain results in death of about 50% of the animals, of which all presenting brain pathology indicative of encephalitis. The proposed role of capsule and adhesion protein in this process is presented.

H2

Viability improvement of three-dimensional cultured skin substitutes by photobiomodulation

Yasuyuki Tsunoi[1], Hiromi Miyazaki[2], Takami Akagi[3], Daizoh Saitoh[2], Mitsuru Akashi[3], and Shunichi Sato[1]
1- National Defense Medical College Research Institute, Division of Bioinformation and Therapeutic Systems, Tokorozawa, Japan; 2- National Defense Medical College Research Institute, Division of Traumatology, Tokorozawa, Japan; 3- Osaka University, Graduate School of Frontier Biosciences, Suita, Japan

In the occurrence of a disaster or terrorism, many victims can suffer severe skin damage or contamination, possibly causing a demand of a large number of skin grafts for critical care. However, there has been a chronic shortage of the stock of allograft skins due to the limited number of donors. Conventional artificial skins and cultured skins have little tolerance to infections due to the lack of vascular networks. We previously developed a three-dimensional (3D) human skin substitute containing vascular networks based on the layer-by-layer (LbL) cell coating technique. However, we faced the problem that the viability of thick 3D skin substitutes was decreased due to the limited nutrient delivery from the circumambient culture medium. In this study, we attempted to apply photobiomodulation (PBM) to improve the viability of cultured tissue. Although the detailed mechanisms of PBM are still under investigation, it is generally accepted that PBM is triggered by the absorption of photons by cytochrome c oxidase in mitochondria, accelerating adenosine triphosphate (ATP) production. We examined the effects of PBM with illumination by a light-emitting diodes (LED) array at four different center wavelengths (440 nm, 523 nm, 658 nm and 823 nm), which were selected based on the absorption spectrum of cytochrome c oxidase. At five days after the start of epidermal differentiation of the 3D skin culture, PBM was applied at each wavelength under the same intensity (15 mW/cm^2) and illumination duration (50 s) conditions. At two days after the illumination, the viability of the 3D skin substitutes (during cultivation) was evaluated by water-soluble tetrazolium (WST) assay. The viability of the skin substitutes (during cultivation) with 823-nm PBM was significantly higher than that without PBM. Although there were no significant differences, the viability of the skin substitutes (during cultivation) with 440-nm PBM was slightly lower, and that with 523-nm or 658-nm was higher, both compared with that without PBM. Since 823-nm light can penetrate into the deeper tissue than the light at other wavelengths, the PBM effect might be obtained for a greater depth region at 823 nm. In conclusion, PBM at 823 nm was effective to improve the viability of the 3D skin substitute during cultivation.

H3

Long- term persistence of humoral immune response against plague in Malagasy patients

Voahangy Andrianaivoarimanana[1], Alice L. Iharisoa[1], Lila Rahalison[1], Marie laurette Ralimanantsoa[2], Maherisoa Ratsitorahina[3], Rado Rakotonanahary[1], Elisabeth Carniel[4], Christian Demeure[4], and Minoarisoa Rajerison[1]
1- Institut Pasteur de Madagascar, Plague Unit, Antananarivo, Madagascar; 2- Ministry of Public Health, Central Laboratory for Plague, Antananarivo, Madagascar; 3- Institut Pasteur de Madagascar, Epidemiology Unit, Antananarivo, Madagascar; 4- Institut Pasteur, Yersinia Research Unit, Paris, France

Plague, a flea-borne zoonotic disease caused by the bacillus, *Yersinia pestis*, still affects resources-limited countries. Humans are occasionally infected through fleabites, thereby causing bubonic plague (BP) or by inhalation of infectious droplets leading to pneumonic plague (PP); the most contagious form. *Y. pestis* expresses a specific capsular F1 antigen which is synthesized in vivo in large quantities. Although anti-F1 IgG are known to be protective against *Y. pestis* infection, information on antibody response to plague infection in human is scarce. As a vaccine preventable disease, knowledge on antibody response is valuable for the development of an effective vaccine to reduce infection rate among exposed population in plague-endemic regions. In this retrospective study, we aim to determine how long humoral immune responses against *Y. pestis* could persist in plague-confirmed patients from Madagascar, the most affected country in the world.

Between 2006 and 2017, 71 recovered plague patients were selected from the national plague database of the Central Laboratory for Plague prior to their recruitment. They were confirmed for plague as *Y. pestis* strain has been isolated from their clinical sample (bubo aspirates or sputum) by bacteriological culture. One single serum was collected from each participant. Negative controls were collected from persons with no plague history in an area of endemicity or in non-endemic plague foci. Serum samples were tested for anti-F1 IgG detection using an ELISA validated for human plague in Madagascar. More than half of the confirmed cases (56%) remained seropositive, with 60 and 40% considered as high- and low-antibody responders, respectively. Anti-F1 IgG antibodies persisted for several years and up to 14.8 years for one individual. Antibody titers decreased over time but were not correlated to time elapsed between the disease onset and serum sampling. In addition, the seroprevalence rate was not significantly different between gender nor age.

Our study highlighted that the serological immune response to F1 antigen may be attributable to individual immune responsiveness and could persist for several years to more than a decade in both BP and PP recovered and confirmed plague patients. However, complementary studies including analyses of the cellular immune response to *Y. pestis* are required for the better understanding of long-lasting

protection and development of a potential vaccine against plague.

H4

Francisella tularensis-one peculiar and possibly emerging pathogen

Valentina Marecic, Ina Kelava, Maša Knezevic, Mateja Ozanic, Mirna Mihelcic, and Marina Šantić
Faculty of Medicine, University of Rijeka, Microbiology and Parasitology, Rijeka, Croatia

Francisella tularensis is a gram-negative intracellular bacterium and the etiological agent of zoonotic and vector borne disease, tularemia in humans and animals. Due to ease of transmission, low infectious dose and severity of infection, subsp. *tularensis* has been classified as Tier 1 select agent. The bacterium has been isolated from more than 250 wild species, including protozoa cells in which *Francisella* could persists for years. There are many species and subspecies of francisela, and many models that have been used for studying this peculiar pathogen. In addition, Francisella uptake and replication occurs in a variety of cell types including phagocytic and non-phagocytic cells. Our studies have showed that different species behave differently in terms of pathogenesis in an *in vitro* and *in vivo* model. Interestingly, francisela exhibits an alteration in the resistance to disinfectants after being growing in amoeba. This overview will summarize the important aspects from ecology of francisela, pathogenesis of tularemia to epidemiology of this possibly emerging pathogen in Europe and worldwide.

H5

[Contribution withdrawn]

H6

[Contribution withdrawn]

H7

Multiple SARS-CoV-2 Mutations in an Immunocompromised Oncology Patient

Gvantsa Brachveli[1], Meri Pantsulaia[1], Ana Papkiauri[1], Giorgi Tomashvili[1], Giorgi Gogoladze[1], Ana Machablishvili[1], Roena Sukhiashvili[1], Gvantsa Chanturia[1], Paata Imnadze[1], Jana Pollakova[2], Michaela Kreitmeier[2], Marcus Antwerpen[2], and Heiner Von buttlar[2]
1- National Center for Disease Control and Public Health, Molecular Epidemiology and Virology, Tbilisi, Georgia; 2- Bundeswehr Institute of Microbiology, Munich, Germany

Throughout the COVID-19 pandemic a number of variants of SARS-Cov-2 have been circulating, some of which might have emerged from immunocompromised hosts. Studies show that persistence of SARS-CoV-2 in such a patient can generate high number of mutations in virus genome. These changes can lead to viral evolution that might have significant impact on the epidemiological level. Here, we report a lethal case of lymphoma diagnosed patient with persistent SARS-CoV-2 infection over at least 75 days.

Two isolates of the infected patient were sequenced: one from December 2020 and the second from March 2021. Both isolates belong to the clade 20B / lineage B.1.1.10 and differ in up to 30 positions distributed over the whole genome from the Wuhan-Hu-1 reference strain. The second isolate accumulated variability of nucleotides within the infection course, which include:

A six nucleotide deletion (143-144del) in the Spike protein causing a loss of two amino acids as well as three additional SNPs resulting in three amino acid alterations - E406D, T470N, E484K. Three more SNPs occurred in ORF1ab gene: One missense mutation (H110Y) and two synonymous mutations (1847C, 4520V).

These findings indicate that some of the mutations observed, e.g., the E484K mutation in the spike protein, could have an impact on the body's immune response and, possibly, on vaccine efficacy. This significant mutation has been found in so called South African - Beta (B.1.351) and Brazilian - Gamma (B.1.1.28) variants. It is important to mention that this mutation has been selected during the prolonged infection and did not originate from interaction with patients infected with Beta/Gamma strains.

In conclusion, our findings suggest that the viral genome can acquire significant changes in immunosuppressed patients with SARS-CoV-2 persistence. Altogether, this study shows the importance of monitoring the SARS-CoV-2 evolution in immunocompromised individuals in order to identify novel, potentially adaptive mutations, and to mitigate the risk of introducing new variants of concern to the community.

Audimax / 11:00 ... 12:30

I New tools for stationary and mobile diagnostics (Part 1 of 2)

Chairs: Katrin Zwirglmaier (DEU) and Heinrich Scheiblauer (DEU)

Session summary

Presentations are online (▭) or on-site (👤) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
I1 👤	**Kilian Stoecker** Munich, DEU	Pulse-Controlled Amplification – a new powerful tool for front-line diagnostics	12+3 min
I2 👤	**Agnieszka Wozniak-Kosek** Warsaw, POL	Using the rapid antigen test for COVID-19 diagnostics in the Hospital Emergency Department of the Military Institute of Medicine in Poland	12+3 min
I3 👤	**Heinrich Scheiblauer** Langen, DEU	Comparative sensitivity evaluation for 122 CE-marked SARS-CoV-2 antigen rapid tests	12+3 min
I4 👤	**Julia T. Klüpfel** Munich, DEU	Rapid detection of (neutralizing) SARS-CoV-2 antibodies by chemiluminescence microarray immunoassay	12+3 min
I5 👤	**Rosina Ehmann** Munich, DEU	A murderer´s diary and portfolio: Different ways of inactivation of SARS-CoV-2 for diverse downstream purposes	12+3 min
I6 👤	**Stephen C. Francesconi** Ft. Belvoir, VA, USA	Development of an inexpensive, disposable, pre-symptomatic agnostic diagnostic of infectious disease to determine fitness for duty and preempt potential for contagious spread	12+3 min

I1

Pulse-Controlled Amplification - a new powerful tool for front-line diagnostics

Kilian Stoecker[1], Katharina Müller[2], Kim Silberreis[1], Maria Weyh[3], Katrin Zwirglmaier[2], and Roman Wölfel[2]

1- Bundeswehr Institute of Microbiology, Dept. for Med. Bio-Reconnaissance & Verification , Munich, Germany; 2- Bundeswehr Institute of Microbiology, Munich, Germany; 3- TU München, Munich, Germany

Molecular diagnostics has become essential in the identification of many infectious diseases, and the detection of nucleic acids often serves as the gold standard technique for most infectious agents. However, established techniques like polymerase chain reaction (PCR) are time-consuming laboratory-bound techniques. Consequently, methods and technologies, which accelerate the identification at the point of care, are becoming more relevant. There are two ways of achieving this aim: by implementing innovative nucleic acid amplification techniques (NAAT) or by using immunochromatographic antigen tests. Here, we present a new NAAT termed pulsed controlled amplification (PCA). PCA is a next generation nucleic acid amplification technology that uses rapid energy pulses to heat microcyclers (micro-scale metal heating elements embedded directly in the amplification reaction) for a few microseconds, thus only heating a small fraction of the reaction volume. The heated microcyclers cool off nearly instantaneously, resulting in ultra-fast heating and cooling cycles during which classic amplification of a target sequence takes place. This reduces the overall amplification time by a factor of up to 10, enabling a sample-to-result workflow in just 15 minutes, while running on a small, battery operated and portable device. We could show that PCA is applicable in the field, without nucleic acid extraction prior to amplification. We furthermore demonstrate that it is suitable for rapid SARS-CoV-2 detection with high sensitivity. Finally, we compared the PCA diagnostic performance to the diagnostic performance of immunochromatographic antigen tests using set of clinical SARS-CoV-2 samples.

I2

Using the rapid antigen test for COVID-19 diagnostics in the Hospital Emergency Department of the Military Institute of Medicine in Poland

Agnieszka Wozniak-Kosek[1], Karol Warda[2], and Ewa Szymczuk[3]
1- Military Institute of Medicine, Laboratory Diagnostics, Warsaw, Poland; 2- Military Institute of Medicine, Hygiene and Epidemiology, Warsaw, Poland; 3- Military Institute of Medicine, Emergency, Warsaw, Poland

The aim of the study was to evaluate the use of a rapid immune-chromatographic antigen test for COVID-19 diagnostics in hospital conditions. The test was assessed on the basis of 4,272 samples of nasopharyngeal swabs taken from patients with COVID-19 symptoms and from contact persons with an infected person who did not show symptoms of infection. The study was conducted from November 2020 to June 2021. During this time, 1,607 COVID-19 + patients were treated at the Military Institute of Medicine. Positive results of the rapid antigen test were obtained for 322 (7.6%) samples taken from HED patients. On the other hand, negative results of the antigen test were obtained for 3927 people, which constituted (91.9%). Pre-laboratory errors were detected in 23 (0.5%) samples and they concerned poorly collected material or too little material. The negative result of the immune-chromatographic test was confirmed by the genetic method using RT-PCR.The correlation between the negative result of the immunechromatographic test and the genetic test was high. Important in the case of COVID-19 + patients staying in the ED is the time to obtain the result, which for antigen tests is very short and is up to 30 minutes from delivery to the laboratory. It is also worth noting that the results obtained for the tested antigen test meet the very high Polish criteria for allowing antigen tests for diagnostic use, i.e. diagnostic sensitivity $\geq$ 90% and diagnostic specificity $\geq$ 97%.The results indicate that the rapid antigen test recommended by WHO in the conditions of the Emergency Department can and should be used for the diagnosis of SARS-CoV-2 infections, especially in patients in the early stage of COVID-19 development, or for patients without typical clinical symptoms.

I3

Comparative sensitivity evaluation for 122 CE-marked SARS-CoV-2 antigen rapid tests

Heinrich Scheiblauer
Paul-Ehrlich-Institut, IVD Testing Laboratory, Langen, Germany

The Paul Ehrlich Institute (Langen, Germany), together with the Robert Koch Institute (Berlin, Germany) and the Institute of Microbiology of the German Armed Forces (München), conducted an independent evaluation of the sensitivity of CE-marked SARS-CoV-2 rapid tests (Ag RDTs) available in Germany to be accepted for reimbursement by the German health care system.

The sensitivity of 122 Ag RDTs was evaluated using a common evaluation panel. The evaluation panel of 50 samples was established by the Robert Koch Institute. Each panel member consisted of pools of up to 10 nasopharyngeal or oropharyngeal swabs with similar SARS-CoV-2 concentrations, determined by RT-PCR as CT and viral RNA copies/ml calibrated against the reference preparation from the German EQA provider INSTAND e. V. In addition, the presence of infectious virus was determined for each panel member. Three viral load panel subgroups were distinguished: CT 17-25 18 pools, CT 25-30 23 pools, CT 30-36 9 pools. A minimum sensitivity of 75% for panel members with CT<25 had to be met to be eligible for listing as reimbursable Ag RDTs.

The results showed that the sensitivity of SARS-CoV-2 Ag RDTs varied over a wide range. Although the sensitivity threshold of 75% for panel members with CT<25 was met by 96 of the 122 tests evaluated, there were also 26 tests with lower sensitivity, some of which failed completely. On the other hand, there were also several tests showing high sensitivity, up to 100% for CT<30.

Overall, based on this evaluation, it was possible to assess the sensitivity of the SARS-CoV-2 Ag-RDTs in direct comparison, thus allowing to distinguish less sensitive from better performing Ag-RDTs. It was also found that swab collection along with sample pretreatment in the respective extraction buffer had a major impact on sensitivity. Another finding was that results around ct 25 and above often showed only faint test lines, requiring experienced personnel for accurate visual reading. In conclusion, market access for SARS-CoV-2 Ag RDT should be based on independent evaluation with minimum sensitivity and specificity requirements.

I4

Rapid detection of (neutralizing) SARS-CoV-2 antibodies by chemiluminescence microarray immunoassay

Julia T. Klüpfel[1], Martin Ungerer[2], Ulrike Protzer[3], Percy Knolle[4], Oliver Hayden[5], Martin Elsner[1], and Michael Seidel[1]
1- Technical University of Munich, Department of Chemistry, Chair of Analytical Chemistry, Munich, Germany; 2- ISAR Bioscience, Planegg, Germany; 3- Technical University of Munich, Institute of Virology, Munich, Germany; 4- Technical University of Munich, Institute of Molecular Immunology, Munich, Germany; 5- Technical University of Munich,

Heinz-Nixdorf-Chair for Biomedical Electronics, Munich, Germany

The SARS-CoV-2 pandemic has kept the world in suspense for more than a year already, claiming many lives and necessitating intensive research to find a way out of it. Even now, with numerous vaccines available that yield hope for the way back to normal life, questions remain: How long does immunity after vaccination last? How effective are vaccines against mutated SARS-CoV-2?

To find answers for these questions, we developed a rapid chemiluminescence microarray immunoassay for the detection of SARS-CoV-2 antibodies on the microarray platform MCR-R. Due to the microarray format with its multiplex capability, antibodies to proteins from different SARS-CoV-2 mutations can be detected simultaneously within a single measurement using low volumes of serum, plasma or whole blood.

The sample is injected into the microarray chip, where antibodies can bind to covalently immobilized SARS-CoV-2 proteins on the polycarbonate chip surface. Subsequently, the chip is flushed automatically with detection antibody and chemiluminescence reagents, the chemiluminescence signal is recorded by a CCD camera and a measurement result is obtained within under five minutes.

Apart from the sole detection of antibodies to SARS-CoV-2, we also extended the immunoassay for the detection of neutralizing antibodies. Therefore, the human receptor for SARS-CoV-2, ACE2, is immobilized on a microarray chip and a mixture of blood sample and SARS-CoV-2 receptor binding domain (RBD) is injected into the chip. Subsequently, the binding of the RBD to ACE2 is measured by chemiluminescence. Depending on the amount of neutralizing antibodies present in the sample, a signal decrease compared to pure RBD can be observed, indicating the ACE2-RBD-binding inhibition strength of the antibodies in the sample. This inhibition is a measure for the ability of the antibodies to prevent cell entry of SARS-CoV-2 and thus to hinder infection. This is a great leap forward, as currently the detection of neutralizing antibodies is a very laborious, time-consuming, and expensive process.

We therefore were able to develop an inexpensive microarray chip for the rapid, automated detection of neutralizing antibodies to SARS-CoV-2 on a novel diagnostic platform which can be of great help for the challenges the world still has to face relating to the COVID-19 pandemic.

I5

A murderer's diary and portfolio: Different ways of inactivation of SARS-CoV-2 for diverse downstream purposes

Rosina Ehmann[1], Katrin Zwirglmaier[2], Mandy Knüpfer[3], Albrecht von Brunn[4], Riccardo De Santis[5], Kilian Stöcker[2], and Roman Wölfel[6]
1- Bundeswehr Institute of Microbiology, Dept. of Virology and Intracellular Pathogens, Munich, Germany; 2- Bundeswehr Institute of Microbiology, Dept. for Med. Bio-Reconnaissance & Verification, Munich, Germany; 3- Bundeswehr Institute of Microbiology, Dept. of Bacteriology and Toxins, Munich, Germany; 4- Max von Pettenkofer Institute, Department of Virology, Munich, Germany; 5- Italian Army Medical Centre, Scientific Department, Rome, Italy; 6- Bundeswehr Institute of Microbiology, Munich, Germany

The omnipresence of the COVID-19 pandemic and the high transmissibility of SARS-CoV-2 has changed our general perception and way of handling respiratory disease agents in the public domain. It also focusses high concentration on a topic that is as evergreen as infectious disease itself: ways to safely inactivate or efficiently reduce the amount of infectious virus.

There are now myriads of ways to physically or chemically deprive a virus of its capability to infect organisms depending on its biological properties. However, the reasons behind the inactivation process may vary significantly which conditions the methods or chemicals used.

While diagnostic units seek simple, fast and complete inactivation of samples for safe and efficient laboratory handling wherever possible, scientists and vaccine manufacturers often require inactivation procedures that carefully maintain certain components like nucleic acid or viral proteins or even the entire particles. Clininicians' needs may vary from total inactivation and safe disinfection of medical equipment and certain areas to patient-friendly and material- or device-compatible ways of reducing surface contamination in the hospital environment. In the field of antiviral drug research, components that safely inactivate virus with minor toxicity on tissues are highly searched for.

Here, we demonstrate our experience with different physical and chemical treatments of SARS-CoV-2 for a potpourri of downstream applications. Heat inactivation proved to be a robust inactivation pretreatment for samples destined for diagnostic nucleic acid amplification tests. Diverse buffer mixtures based on detergents were tested for their capacity to inactivate virus while serving at the same time as PCR- or rapid test compatible sam-

ple buffers. Betapropiolactone was found to be a reliable inactivating agent with highly protein- and nucleic acid-structure retaining properties. BPL-inactivated virus material proved useful for validation of antigenic tests and for easily shippable sample materials in external quality assessments. It was even successfully used for the preparation of training materials for scent detection dogs for the screeining of COVID-19 in people. Lastly, an LED device with a special combination of different interacting wavelengths was found to be an integrative tool to reduce surface contaminations with SARS-CoV-2 without harming sensitive materials or tissues where UV irradiation cannot be applied.

I6

Development of an inexpensive, disposable, pre-symptomatic agnostic diagnostic of infectious disease to determine fitness for duty and preempt potential for contagious spread

Stephen C. Francesconi[1], Nicholas Meyerson[2], Sara Sawyer[3], and Richard Schoske[1]
1- Defense Threat Reduction Agency, Chem Bio Defense, Ft. Belvoir, VA, USA; 2- Darwin Biosciences, Boulder, USA; 3- University of Colorado, Boulder, USA

For many reasons, it would be useful to know in advance one was going to get sick. Consider the sailor preparing to board a submarine that feels well but has contracted a respiratory virus. His asymptomatic infection could potentially put the entire crew at risk. Think too of the aircrew or platoon member that is exposed and incubating the pathogen. The mission they are on may have already begun once they show symptoms and become infectious to the detriment of themselves and their teammates. Moving the diagnostic window to include days prior to symptoms will give the warfighter confidence he or she will be fit to complete the mission. A rapid test could quickly mark those that need to seek further medical attention despite feeling healthy that morning.

DTRA recently began an Other Transaction Agreement with Darwin Biosciences in Boulder, Colorado to develop "SickStick". The prototype device will be an inexpensive, disposable, saliva based diagnostic that measures biomarkers present in an exposed patient's saliva before they present symptoms. Preliminary studies have shown at least 15 mRNAs appear days before symptoms. The company is currently down selecting the markers to correlate with actual infections. The goal is to couple a fresh saliva sample with isothermal amplification and visualize the result on a lateral flow membrane. If successful at prognostic identification of those that will become sick, this will be of tremendous advantage to the Department of Defense. Preliminary estimates hope to drive the cost to under $3.00 per test.

Preliminary data came from COVID-19 infected students at the University of Colorado. The diagnostic biomarkers were able to determine accurately which students were asymptomatic. Conventional PCR testing confirmed their infection, allowing them to quarantine and avoid infecting others. While SARS-CoV-2 in patients is unusual in shedding prior to showing symptoms, and up to fifty percent of patients may stay asymptomatic but become infectious. One or two days notice of an impending infection may be enough to prevent a "super spreader" scenario on a navy vessel, a commercial flight, or any large gathering of people. Further testing may allow treatment to begin prior to symptom appearance, for example beginning Tamiflu in cases of confirmed influenza.

Garden Hall / 11:00 ... 12:30

J

The dose makes the poison: An update on the detection of biological toxins

Chairs: Mandy Knüpfer (DEU) and Brigitte G. Dorner (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
J1 🧍	**Brigitte G. Dorner** Berlin, DEU	European programme for the establishment of validated procedures for the detection and identification of biological toxins (EuroBioTox)	20+5 min
J2 🧍	**Andreas Auernhammer** Munich, DEU	Development of a multiplex algae toxin immunoassay for the monitoring of algal blooms in surface water	12+3 min
J3 🧍	**Jiri Dresler** Prague, CZE	In-situ Detection of Botulinum Neurotoxin A by MALDI Mass Spectrometry Using Functionalized Chips	12+3 min
J4 🖵	**Maren Krüger** Berlin, DEU	A multiplex biochip for fast and sensitive detection of eight biotoxins for differential diagnosis of ricin intoxications	12+3 min
J5 🧍	**Sylvia Worbs** Berlin, DEU	Differentiation, Quantification and Identification of Abrin and *Abrus precatorius* Agglutinin	12+3 min

J1

European programme for the establishment of validated procedures for the detection and identification of biological toxins (EuroBioTox)

Sylvia Worbs[1], Bettina Kampa[1], Martin Skiba[1], Katrien Busschots[4], Reinhard Zeleny[4], Julien Masquelier[5], Anne Puustinen[6], Paula Vanninen[6], Christine Rasetti-Escargueil[7], Michel Popoff [7], Emmanuel Lemichez[7], Anne-Sophie Mierzala[8], Hervé Volland[8], François Becher[8], Stéphanie Simon[8], Berivan Boran[9], Yacine Nia[9], Jacques-antoine Hennekinne[9], Jasmin Weisemann[10], Daniel Jansson[11], Marc-andré Avondet[12], Werner Luginbühl[13], Roland Josuran[14], Christiane Zaborosch[14], Luke Burns[15], Katrina Campbell[15], and Brigitte G. Dorner[1]

1- Robert Koch Institute, Centre for Biological Threats and Special Pathogens / Biological Toxins, Berlin, Germany; 4- European Commission, Joint Research Centre, Geel, Belgium; 5- Sciensano, Tervuren, Belgium; 6- University of Helsinki, Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Helsinki, Finland; 7- Institut Pasteur, Département de Microbiologie, Bacteries Anaerobies et Toxines, Paris, France; 8- CEA-SACLAY, Laboratoire d'Etudes et de Recherche en Immunoanalyse, Gif sur Yvette, France; 9- ANSES, Food Safety Laboratory, SBCL Unit, Maisons-Alfort, France

Biological toxins are known as causative agents of food poisoning, but some of them also have a history as warfare agents and could be used in a bioterrorism context. Previous studies showed that there is a lack of robustness in European preparedness for biotoxin incidents. There is a need for standard analytical tools and procedures, reference materials, state-of-the-art training and establishment of a European proficiency testing scheme.

EuroBioTox is a Horizon 2020 project integrating 13 consortium partners and 50 network partners from 23 countries from the health, food, military and verification sectors. The project aims at establishing a Pan-European network of competence for the analysis of biological toxins of potential bioterrorism threat (1). The toxins in the scope of EuroBioTox comprise selected large protein toxins (ricin, abrin, botulinum neurotoxins [BoNT], staphylococcal enterotoxin B [SEB]) as well as small molecule biotoxins (saxitoxin [STX]).

Using current best practice, the EuroBioTox core members develop and validate improved analytical tools, reagents and standard operating procedures based on realistic incident scenarios. Alternative and more accurate *in vitro* tests for the ethically questionable animal test for BoNT are under evaluation.

Progress beyond state of the art has been achieved by production of five candidate reference materials for different biological toxins to be certified after the current comprehensive molecular characterisation.

Training courses at basic and advanced levels have been conducted within the network tailored to the different methods and toxins, followed by a series of proficiency tests (currently five) to disseminate best practice methods across Europe. The creation of a European repository with proprietary toxin-specific tools was initiated to harmonise detection methods. With respect to the specific needs of first responders, new conceptual guidelines on sampling, detection and decontamination were established focusing on biological toxins.

EuroBioTox is implementing a comprehensive mechanism of training, method sharing, improvement of quality assurance measures and proficiency testing. It is expected that the spreading of good analytical practices will improve preparedness and response planning at national and international level.

Reference: https://eurobiotox.eu">https://eurobiotox.eu

J2

Development of a multiplex algae toxin immunoassay for the monitoring of algal blooms in surface water

Andreas Auernhammer[1], Cindy Dongxin hu[2], and Michael Seidel[1]
1- Technical University Munich, Institute of Hydrochemistry, Munich, Germany; 2- A.U.G. Signals Ltd., Environmental Monitoring & Quality Control, Totonto, Canada

Algal blooms, the increased growth of cyanobacteria, have been observed more frequently in recent years. This phenomenon is favored by climate change and the eutrophication of water bodies. During an exponential growth spurt of cyanobacteria, the contamination of water bodies with cyanotoxins increases. Cyanotoxins poses a threat to the affected ecosystem as well as to human health. To prevent accidents, comprehensive monitoring of potentially endangered waters is urgently needed.

In cooperation with AUG Signals, Canada, an online monitoring system with cloud-based data processing is developed as early warning system in order to be able to predict increased cyanobacterial growth and algal blooms at an early stage. The online monitoring system is designed to collect information on certain parameters that can lead to excessive growth of cyanobacteria and to evaluate them using an online cloud-based data management system. Part of the project is the TRITON water sensor system prototype designed from AUG Signals, which can provide data of the surface water samples collected at selected sites or can be set up on-site to collect real-time data from water bodies. The system has two primary components, including an optical sensor module providing an UV-VIS absorption spectral analysis, ranging from 200 to 850 nm and a sonde hosting pH, temperature, conductivity, TDS and TSS, turbidity, dissolved oxygen and nitrate and total nitrate.

This sensor system is combined with the a fully automated indirect competitive flow-through microarray immunoassay for the simultaneous detection and quantification of different cyanotoxins with automated sample preparation and enrichment steps for free and intracellular toxins. The immunoassay is performed on the Microarray Chip Reader (MCR R) in less than 7 minutes. For microcystin-LR, for example, the detection limit is 4.8 µg/L. Lower toxin concentrations can be detected with the help of immunomagnetic separation. It can also be used to quantify intracellular toxins without the negative influence of cellular- or matrix components on the immunoassay.

The early warning system will be able to collect and process multiple sensor data in parallel. Surface water is monitored continuously by the TRITON water sensor. Significant changes for key parameters will identify the generation of algae blooms and the MCR R will confirm the increase of cyanotoxins. Therefore, action instructions for a better risk assessment are possible.

J3

In-situ Detection of Botulinum Neurotoxin A by MALDI Mass Spectrometry Using Functionalized Chips

Jiri Dresler[1], Petr Pompach[2], Petra Darebna[3], Zuzana Kalaninova[3], and Petr Novak[3]
1- Military Health Institute, Prague, Czech Republic; 2- Institute of Microbiology, v.v.i., Czech Academy of Sciences, Prague, Czech Republic; 3- Institute of Microbiology, v.v.i., Prague, Czech Republic

Botulinum neurotoxins (BoNTs) are bacterial proteins produced by Clostridium species causing lethal disease botulism. The widely used test for the identification of BoNTs in both clinical specimens and food is the mouse bioassay, which suffers by several limitations. Thus, there is a need for a rapid and sensitive method for detecting of these BoNTs. Matrix assisted laser desorption (MALDI) mass spectrometry in combination with in vitro enzymatic assay Endopep-MS was recently demonstrated as a robust and fast technique for detection of BoNTs. In this study, we follow up this idea and use specifically designed peptide substrate biotinylated at both termini as a target for BoNT A. The products of the enzymatic reaction are

peptide fragments of the original substrate that are detectable by MALDI mass spectrometry. We used MALDI chips functionalized with biotin-binding proteins streptavidin, neutravidin and avidin to enrich the biotinylated peptide fragments from crude biological matrices. These chips were prepared by modification of indium tin oxide glass using ambient ion soft landing under atmospheric pressure. One microliter of the sample after the specific BoNT A enzymatic reaction was applied on the MALDI chip. After incubation and washing the whole MALDI chips in buffer, each spot was covered by CHCA matrix. The resulting peptide fragments were measured by Autoflex MALDI mass spectrometer. The functionalized MALDI chips achieved low nonspecific interactions and efficient peptides ionization to detect BoNT A in samples. Two peptides, products of the enzymatic reaction, were observed in the spectra. The limit of detection for enriched peptides was 0.01 ng/ml of BoNT A concentration. The results indicate that detection of BoNT A using functionalized MALDI chips is sensitive, robust, fast and might be automated for general use in MALDI Biotyper system equipped laboratories.

J4

A multiplex biochip for fast and sensitive detection of eight biotoxins for differential diagnosis of ricin intoxications

Maren Krüger[1], Thea Neumann[1], Stefan Mahrhold[2], Jasmin Weisemann[2], Christopher Pöhlmann[3], Daniel Stern[1], Martin B. Dorner[1], Thomas Elßner[3], Andreas Rummel[2], and Brigitte G. Dorner[1]
1- Robert Koch Institute, Centre for Biological Threats and Special Pathogens / Biological Toxins, Berlin, Germany; 2- Hannover Medical School, Institute for Toxicology, Hannover, Germany; 3- Bruker Daltonik GmbH, Leipzig, Germany

Biological toxins may cause severe medical conditions in humans. Due to their accessibility and efficacy, they can and have been prepared by laypersons with malevolent intent to harm individuals or groups of people. One of the toxins which aroused attention in the recent past is the ribosome-inactivating plant toxin ricin, which causes bloody diarrhoea as cardinal symptom after oral uptake, along with other rather unspecific symptoms due to its cytotoxicity.

Other cytotoxins, which mainly play a role in natural intoxications, may cause similar symptoms and must therefore be considered in the differential diagnosis for alleged ricin intoxications. Among those are the structurally and functionally related plant toxin abrin, the large glycosylating toxins A and B from *Clostridioides difficile* (TcdA and TcdB), the pore forming *Clostridium perfringens* enterotoxin (CPE) and epsilon toxin (Etx), as well as ribosome-inactivating shiga toxins 1 and 2 from *Escherichia coli* (Stx1 and Stx2).

In the framework of the BMBF-funded project SensTox, we explored newly generated or commercially available monoclonal antibodies in comparison to innovative application of the endogenous receptors, as undressed proteins or functionalised in nanodiscs, for the detection of the biotoxins. The reagents were analysed regarding their kinetic binding properties by surface plasmon resonance measurements and for specificity and sensitivity in ELISA. While the innovative approach was successfully realised for a subset of the toxins, we determined that this application is not generally transferable to all types of receptors tested in this study. In addition to the receptor-based assays, we were able to establish detection assays for all of the toxins based on monoclonal antibodies. The most sensitive assays were transferred to the electrical biochip-based platform called portable BioDetector integrated (pBDi, Bruker Daltonik GmbH) and analysed for their applicability in the multiplex format.

Here, we present a multiplex detection chip based on highly affine monoclonal antibodies, which allows sensitive detection of biotoxins abrin, ricin, TcdA, TcdB, Etx, CPE, Stx1 and Stx2 in the low ng/ml-range from one sample within 20 minutes. This represents a valuable and rapid support for diagnostics of diseases that are relevant for differential diagnosis of ricin intoxication.

J5

Differentiation, Quantification and Identification of Abrin and *Abrus precatorius* Agglutinin

Sylvia Worbs[1], Bettina Kampa[1], Martin Skiba[1], Eva-Maria Hansbauer[1], Daniel Stern[1], Hervé Volland[2], Francois Becher[2], Stéphanie Simon[2], Martin B. Dorner[1], and Dorner B. Dorner[1]
1- Robert Koch Institute, ZBS3, Berlin, Germany; 2- CEA, Département Médicaments et Technologies pour la Santé, Gif-sur-Yvette, France

The shrub *Abrus precatorius* belongs to the *Fabaceae* family and is also known as jequirity bean, crab's eye, rosary or paternoster pea plant. The seeds contain the highly toxic lectin abrin which has gained considerable interest in the recent past due to its potential malevolent use. However, reliable and easy-to-use assays for the detection and discrimination of abrin from related plant proteins such

as *Abrus precatorius* agglutinin or the homologous toxin ricin from *Ricinus communis* are sparse. To address this gap, we generated a panel of highly specific monoclonal antibodies against abrin and the related *Abrus precatorius* agglutinin.

These antibodies were comprehensively characterised using surface plasmon resonance as well as other techniques and provided the basis to develop methods for the detection of abrin and *A. precatorius* agglutinin from different matrices.

We established two sandwich ELISAs to preferentially detect abrin or *A. precatorius* agglutinin (limit of detection 22 pg/mL for abrin; 35 pg/mL for *A. precatorius* agglutinin). Furthermore, an abrin-specific lateral flow assay was developed for rapid on-site detection (limit of detection 1 ng/mL abrin). These assays were validated for complex food, environmental and clinical matrices illustrating broad applicability in different threat scenarios. Additionally, the antibodies turned out to be suitable for immuno-enrichment strategies followed by mass spectrometry-based approaches for unambiguous identification.

Finally, in a real case of attempted suicide by oral *A. precatorius* ingestion, the ELISA and MS methods were successfully applied to confirm abrin poisoning from fecal samples. To the best of our knowledge, this is the first case where ELISA-based detection and quantification as well as LC-ESI-MS/MS-based identification were successfully implemented for abrin detection in a real-life case of human *A. precatorius* intoxication.

Reference: Worbs, S., et al., Differentiation, quantification and identification of abrin and Abrus precatorius agglutinin. Toxins, 2021 Apr 18;13(4):284.

Audimax / 13:30 ... 15:30

K New tools for stationary and mobile diagnostics (Part 2 of 2)

Chairs: Kilian Stoecker (DEU) and Benedict Gannon (GBR)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
K1 🧍	**Jan Baumann** Copenhagen, DNK	Classification and Standardization of Rapid Response Mobile Laboratories	12+3 min
K2 🧍	**Veronika Merold** Munich, DEU	Development and Validation of rRNA Targeted Reverse Transcription Real Time PCR Assays for the Sensitive and Rapid Diagnostics of High Consequence Pathogens	12+3 min
K3 🖵	**Lovasoa N. Randriantseheno** Antananarivo, MDG	Rapid and efficient detection of *Yersinia pestis* in plague biological samples using the loop-mediated isothermal amplification	12+3 min
K4 🧍	**Gregor Grass** Munich, DEU	A unique single nucleotide polymorphism in multi-copy 16S rRNA genes and transcripts facilitates sensitive identification of *Bacillus anthracis* by real time PCR	12+3 min
K5 🧍	**Mostafa Bentahir** Brussels, BEL	Nucleic acids isothermal amplification methods for fast and reliable detection of biothreat agents: potential for on-site use in operational conditions	12+3 min
K6 🖵	**Aleksandr S. Safatov** Novosibirsk, RUS	The use of biochips with SOI-FET sensors for the indication of viruses in aerosol samples	12+3 min

K1

Classification and Standardization of Rapid Response Mobile Laboratories

Jan Baumann
WHO, Copenhagen, Denmark

[Abstract pending]

K2

Development and Validation of rRNA Targeted Reverse Transcription Real Time PCR Assays for the Sensitive and Rapid Diagnostics of High Consequence Pathogens

Veronika Merold, Kim Silberreis, and Kilian Stoecker
Bundeswehr Institute of Microbiology, Dept. for Med. Bio-Reconnaissance & Verification , Munich, Germany

Real-time PCR has become an essential tool in clinical microbiology and is been used for the acute diagnostics of many pathogens. Key performance indicators of real-time PCR assays are their specificity as well as their analytical and clinical sensitivity. One way to maximize the sensitivity of such diagnostic real-time PCRs is the use of genomic targets, which are present in several copies in the target cells. Here we use the naturally preamplified ribosomal RNA as target for specific and highly sensitive reverse-transcription real-time PCR detection of two high consequence pathogens, *Yersinia pestis* and *Francisella tularensis*. We determined their analytically sensitivity and illustrate that the newly designed assays are superior compared to other previous published real-time PCR assays. Furthermore, we used spiked clinical sample matrices to evaluate their clinical applicability. Finally, we demonstrate that these assays can be applied on heat-inactivated samples without the need of time-consuming nucleic acid extraction.

K3

Rapid and efficient detection of *Yersinia pestis* in plague biological samples using the loop-mediated isothermal amplification

Lovasoa N. Randriantseheno[1], Anjanirina Rahantamalala[2], Ando Randrianierenana[3],

Minoarisoa Rajerison[1], and Voahangy Andrianaivoarimanana[1]
1- Institut Pasteur de Madagascar, Plague Unit, Antananarivo, Madagascar; 2- Institut Pasteur de Madagascar, Immunology of Infectious Diseases Unit, Antananarivo, Madagascar; 3- University of Antananarivo, Department of Applied and Fundamental Biochemistry, Antananarivo, Madagascar

Plague, *Yersinia pestis* infection, represents a serious public health problem in Madagascar where 75% of the human cases worldwide are reported. Its pneumonic form is particularly contagious because of the possibility of human-to-human transmission. As the current Covid-19 pandemic reminds us, rapid and reliable diagnostic tools are very important to monitor, control and tackle the spread of infectious diseases. In terms of plague confirmation, *Y. pestis* strain isolation remains the gold standard according to the World Health Organization. However, it is laborious and time consuming. Serology and molecular detection of *Y. pestis* specific genes (*caf1*) are faster but only available at the Central Laboratory for Plague in Antananarivo. Rapid diagnostic test for F1 antigen detection (F1RDT) is currently the only available point-of-care test but is not confirmatory when used alone. Here, we report the development of loop-mediated isothermal amplification (LAMP) for *Y. pestis* detection.

Primers were designed to target the *caf1* gene. Optimization of temperature, reaction time and reagents was performed using *Y. pestis* DNA extracts and Sybr Green I color change was used for results visualization. The optimized procedure was evaluated for cross-reaction against 14 other pathogens and the detection limit was determined by 10-fold dilution of *Y. pestis* DNA extracts. Hundred and thirteen plague biological samples and 47 non-plague samples were tested to assess the sensitivity and specificity of LAMP *caf1*. Efficient detection was obtained with 0.95 M betaine after 35 minutes reaction at the constant temperature of 63°C maintained with water bath. No cross-reaction was detected against the 14 pathogens tested and the detection limit was as low as 3.79 pg/µl. Compared to the gold standard, LAMP *caf1* had a sensitivity of 97.9% (95% CI: 89.1%-99.9%) and a specificity of 94.6% (95% CI: 88.6%-97.9%) with a kappa coefficient of 0.89 showing an almost perfect agreement between the two tests.

In conclusion, LAMP *caf1* is rapid and relatively inexpensive, requiring only a water bath. The technique is also simple since results are directly readable with the naked eye. It can detect *Y. pestis* in human plague biological samples with high sensitivity and specificity. Therefore, it can be used for rapid diagnosis and confirmation of plague during outbreaks in combination with F1RDT in resource limited countries such as Madagascar where plague is still endemic.

K4

A unique single nucleotide polymorphism in multi-copy 16S rRNA genes and transcripts facilitates sensitive identification of *Bacillus anthracis* by real time PCR

Peter Braun, Martin D.-T. Nguyen, and Gregor Grass
Bundeswehr Institute of Microbiology, Bacteriology and Toxinology, Munich, Germany

Introduction: Among the *Bacillus* species, the anthrax pathogen *Bacillus anthracis* poses the greatest risk to human and animal health. Identification of *B. anthracis* by polymerase chain reaction (PCR) or other methods is challenging because of the bacterium's close genetic relationship to other species of the *Bacillus cereus sensu lato group* (such as *Bacillus cereus* or *Bacillus thuringiensis*). Thus, molecular detection and identification is depending on the use of a limited set of species-specific gene targets (e.g., *dhp61* or *PL3*) or unique single nucleotide polymorphisms (SNPs) (e.g., in *rpoB* or *plcR* genes). All of these represent single-copy targets within a *B. anthracis* genome equivalent. Here, we made use of an *in silico* validated multi-copy target, a species-specific SNP. This SNP is conserved and present in up to five loci of the 16S rRNA gene sequences in every *B. anthracis* genomic data analyzed to date (n=959).

Methods: From this knowledge-base, a hydrolysis probe-based PCR assay was developed and experimentally validated. In this assay, the *B. anthracis* specific SNP is interrogated for by a fluorescently-labeled probe; the alternative SNP-state is masked by a fluorescently dark "competitor" probe. In an effort to push the detection limit, the assay was further adapted for reverse transcription PCR targeting 16S rRNA transcripts. These transcripts are found in cells in concentrations 3-4 log units higher than multi-copy 16S rRNA operons. Thus, the assay targets the SNP in genomic DNA and RNA employing identical primers and probes.

Results: The assay was specific as only *B. anthracis* DNA or RNA yielded positive results. DNA detection performed linear over 9 log10 units and was sensitive with a limit of detection (LoD) of 2.9 copies/reaction (i.e., about one genome equivalent). For *B. anthracis* 16S rRNA the assay was also specific and linear over 8 log10 units with a LoD of 6.3 copies/reaction.

Discussion: In this study we introduced the first

combined chromosomal DNA- and ribosomal RNA-targeting multi-copy real time PCR assay for *B. anthracis*. Harnessing unique SNPs in 16S rRNA genes and their transcripts strongly highlights the great potential that such genomic variations have not only for identification of *B. anthracis* and for diagnostics of anthrax disease. Potentially this approach is also applicable to other pathogens which are otherwise difficult to discriminate from their less notorious relatives.

K5

Nucleic acids isothermal amplification methods for fast and reliable detection of biothreat agents: potential for on-site use in operational conditions

Mostafa Bentahir[1], Pierre Vandenberghe[2], Leonid Irenge[3], and Jean-luc Gala[4]
1- Belgian Armed Forces, Defense Laboratories Department (DLD), Biothreats unit, Brussels, Belgium; 2- Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, 1. Centre de Technologies Moléculaires Appliquées, Brussels, Belgium; 3- Belgian Armed Forces, Defense Laboratories Department (DLD), Biothreats unit, Belgium; 4- Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Centre de Technologies Moléculaires Appliquées, Belgium

The speed, sensitivity and selectivity of isothermal amplification methods make them an interesting alternative to real-time PCR-based methods for on-site microbial identification in a context of a natural outbreak, accidental or deliberate release. Through a series of applications, we illustrate here after the use of the recombinase polymerase amplification (RPA) assays and loop-mediated isothermal amplification (LAMP). A RPA test was developed and validated for rapid on-site screening and identification of *Bacillus anthracis* (BA), using the adenylate cyclase gene (*adk*), which is conserved in the *Bacillus cereus* group, and three BA-specific targets (BA_5345 chromosomal marker, lethal factor *lef* [from pXO1], and capsule-biosynthesis-related *capA* [from pXO2]). LAMP tests were was developed to identify the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), epidemic *Vibrio cholerae* strains, and the vector-borne viral pathogens yellow fever (YFZ), zika (ZIKV)and dengue type 1-4 (DENV). For SARS-CoV-2, the E (envelope)-gene was chosen. For SARS-CoV-2, the LAMP was successfully compared to standard q-PCR assays on 400 SARS-CoV-2-positive nasopharyngeal swabs. A duplex LAMP using E and S (spike) genes was also successfully tested using the HybriDetect 2T lateral flow membrane. A triplex LAMP assay was developed to identify YFV, ZIKV and DENV and tested on a panel of 41 reference viral and bacterial strains (https://www.beiresources.org/); in addition, the LAMP-amplicons were labelled with a fluorophore and biotin moiety and then detected on a lateral flow membrane spotted with antibodies to the fluorophore in the detection zone, and containing streptavidin-nanogold particles in the conjugate pad. Three simplex LAMP assays were developed to screen for *Vibrio cholerae* using specific genes (cholera toxin (ctx$A$), cholera toxin co-regulated pilus (*TcpA),* and outer membrane protein (*OmpW*)). These assays are currently being validated on a panel of 150 *Vibrio* strains. In the current study, we have also tested a rapid nucleic acids extraction protocol based on a lysis buffer and a temperature treatment at 95°C for 5 min (Lucigen). This fast extraction protocol yielded comparable results to standard viral RNA and bacterial DNA extraction methods. Overall, our results confirm the high efficiency of isothermal amplification compared to PCR and its potential for on-site use under operational conditions.

K6

The use of biochips with SOI-FET sensors for the indication of viruses in aerosol samples

Aleksandr S. Safatov[1], Vladimir M. Generalov[1], Anastasia A. Cheremiskina[1], Galina A. Buryak[1], Olga V. Naumova[2], Elza G. Zaitseva[2], and Alexander L. Aseev[3]
1- FBRI State Research Center of Virology and Biotechnology "Vector" of Rospotrebnadzor, Department of biophysics and ecological researches, Novosibirsk, Russian Federation; 2- A.V. Rzhanov Institute of Semiconductor Physics, Siberian Branch of the Russian Academy of Sciences, Laboratiry of silicon microelectronics' technology, Novosibirsk, Russian Federation; 3- A.V. Rzhanov Institute of Semiconductor Physics, Siberian Branch of the Russian Academy of Sciences, Department of Physics and Technology of Low-Dimensional Semiconductors, Micro - and Nanostructures, Russian Federation

Express indication of viruses in aerosol samples is an urgent task for emergency response to possible threats. The use of PCR, ELISA and other methods requires sample analysis time in excess of 5 minutes. In addition, the sensitivity of these methods is insufficient for indication very low concentrations of viruses in samples. The use of biochips with SOI-FET sensors for the indication of viruses in aerosol samples can reduce the analysis time to less than 3 minutes and achieve sensitivity at the level

of femtomole virus concentrations in samples.

The Institute of Semiconductor Physics SB RAS has developed a biochip design with SOI-FET sensors. Their performance has been investigated and published using the following model systems.

1. Ebolavirus antibodies on the surface of the nanoribbon + Ebolavirus VP40 protein [1].
2. Vaccinia virus antibodies on the surface of the nanoribbon + vaccinia virus [2].
3. Antibodies to influenza A virus on the surface of the nanoribbon + influenza A virus [3].
4. Antibodies to SARS CoV-2 on the surface of the nanoribbon + pseudoviral particles with SARS CoV-2 protein S [4].

The experimental results obtained prove that in the above model systems, indication times of less than 3 minutes are achieved, and the sensitivity of the sensors reaches the femtomole concentrations of viruses in the samples. Thus, the use of a biochip with SOI-FET sensors is promising for indication very low concentrations of viruses in aerosol samples.

References:

1. Generalov V.M., et al. (2019). Detection of Ebola Virus VP40 Protein using a Nanowire SOI Biosensor // Optoelectronics, Instrument. Data Process., 55(6) 618-622. DOI 10.3103/S875669901906013X.
2. Generalov V.M., et al. (2021). Identifying the Vaccinia Virus with the Use of a Nanowire Silicon-on-Insulator Biosensor // Optoelectronics, Instrument. Data Process., 57(1) 37-43. DOI: 10.3103/S8756699021010040.
3. Malsagova K.A., et al. (2021). Detection of Influenza Virus Using a SOI-Nanoribbon Chip, Based on an N-Type Field-Effect Transistor // Biosensors, 11(4), 119. DOI 10.3390/bios11040119.
4. Generalov V., et al. (2020). Indication of the Coronavirus Model Using a Nanowire Biosensor // Proceedings, 60(1), 50. https://doi.org/10.3390/IECB2020-07228

Garden Hall / 13:30 ... 15:30

L

Dual-use-research of concern

Chairs: Roman Wölfel (DEU) and Isabel Hunger-Glaser (CHE)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
L1 🖵	**Filippa Lentzos** London, GBR	History and Politics of DURC Governance	25+5 min
L2 🧍	**R. Guy Reeves** Plön, DEU	Self-spreading vaccines in the environment: An innovation or the erosion of an evidence-based norm?	20+5 min
L3 🖵	**Thomas Lengauer** Saarbrücken, DEU	Responsible handling of security-relevant research in Germany	20+5 min
L4 🧍	**Svenja Vinke** Bielefeld, DEU	The biosecurity education gap: Most life-sciences researchers have never heard of the term "dual use research"	15+5 min

L1

History and Politics of DURC Governance

Filippa Lentzos
King's College London, Centre for Science & Security Studies (CSSS), London, United Kingdom

This talk provides an overview of the history and politics of DURC governance. It traces the evolution of dual-use concepts, and the introduction of the term "dual use of concern" in relation to security concerns arising from the life sciences. It then presents international tools and governance mechanisms to address DURC concerns, highlighting how few countries have any enforceable standards in this area, before briefly elaborating different frameworks for assessing security concerns. The talk argues for developing international standards on research and development activities that can be re-purposed to cause harm.

L2

Self-spreading vaccines in the environment: An innovation or the erosion of an evidence-based norm?

R. Guy Reeves
Max Planck Institute for Evolutionary Biology, Department of Evolutionary Genetics, Plön, Germany

Self-spreading genetically altered viruses are too unstable to be used safely and predictably outside contained facilities in the environment; this can arguably be said to broadly state a longstanding evidence based norm. It was this norm, rather than technical limitations, that restricted past periodic efforts to promote or assemble self-spreading vaccines. I will examine the specifics of a current cycle of interest in self-spreading vaccines and whether the promoted upsides are achievable within current (or any conceivable) vaccine regulatory system. Questions about the role that military funding is playing in erosion of norms in biological research will also be briefly considered.

L3

Responsible handling of security-relevant research in Germany

Thomas Lengauer
Max Planck Institute for Informatics, Computational Biology, Saarbrücken, Germany

Scientific freedom is a fundamental requirement for ensuring progress and is therefore enshrined in the German Basic Law. Yet in almost all scientific disciplines free research is associated with risks, primarily resulting from the danger of useful research findings being misused, for example as weapons of war and means of criminal or terrorist activities.

Legal provisions offer a limited means of controlling the opportunities and risks associated with free research. Research methods and directions are constantly changing and research findings, as well as their future application, tend to be almost impossible to predict. The German National Academy of Science Leopoldina and the German Research Foundation (DFG) work steadily to ensure that

ethical principles and mechanisms for the responsible handling of free research and research risks are developed within the scientific community. The two organisations published a general code of conduct (CoC) on handling security-relevant scientific research [1] which is aimed primarily at the government-funded research sector. It has been widely adapted throughout Germany placing great importance on instruments of self-governance within the scientific community. The advantage of self-governance lies in researchers' high level of expertise regarding the relevant subject and the fact that it allows for a flexible response.

In order to support the sustainable implementation of the CoC, in the year 2014 Leopoldina and DFG established the Joint Committee on the Handling of Security-Relevant Research, consisting of 12 scientific experts in relevant disciplines. This Committee stays abreast of the progress in enacting the CoC through monitoring and provides support to the research institutes for the implementation of the recommendations. Furthermore, it serves as a contact point for questions and a platform for the bundled exchange of information. In line with the CoC, more than 90 local committees for ethics in security-relevant research have been established throughout Germany [2]. These committees enable the individual institutes to responsibly deal with troubling issues arising from research projects and decide themselves how to continue.

References:

1. www.leopoldina.org/uploads/tx_leopublication/2014_06_DFG-Leopoldina_Scientific_Freedom_Responsibility_EN.pdf
2. https://www.leopoldina.org/en/about-us/cooperations/joint-committee-on-dual-use/dual-use-progress-reports/

L4

The biosecurity education gap: Most life-sciences researchers have never heard of the term "dual use research"

Svenja Vinke[1] and Irina Rais[2]
1- Center for Biotechnology (CeBiTec), Bielefeld University, Biology, Bielefeld, Germany; 2- University of Cologne, Biochemistry, Cologne, Germany

For a strong biosecurity threat prevention network, education of life sciences students on dual use issues plays a crucial role. Today's students will be tomorrow's researchers and industry leaders, but teaching biosecurity issues is a very underrepresented part in their university education and even many later career scientists are unaware of the importance of considering biosecurity risks. The purpose of the presented study was to examine the state of knowledge about dual use research among life sciences students and to test an alternative teaching approach for communicating the importance of considering biosecurity risks in the biggest synthetic biology competition, the international Engineered Machine (iGEM) competition. The results from the workshop and the survey show that educational machinery so far failed to integrate teaching about these issues. For example, only 41 % of the participants knew what dual use in the context of science means and 76 % want a more pronounced education on dual use issues. Throughout the past years, implementing new biosecurity teaching concepts has not been a priority. A big maladministration we need to change as soon as possible. While we do so, we need to incorporate teaching about non-pathogen related dual use research since current regulation strategies for non-pathogen related dual use research rely almost entirely on self-regulation and thus awareness of these issues. To improve an entire educational machinery, we need to bring all actors together and invest resources into new educational projects to ensure that life sciences research of today and the future is conducted in a safe and responsible way.

Audimax / 16:00 ... 18:00

M

Antimicrobials and vaccines

Chairs: Gerd Sutter (DEU) and Ruth Brack-Werner (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
M1 🧍	**Gerd Sutter** Munich, DEU	Vaccination against COVID-19 - the development of candidate vaccine MSARSCoV2	20+4 min
M2 🧍	**Ariane Volkmann** Martinsried, DEU	An MVA-based WEVEE vaccine protects mice against all 3 equine encephalitis viruses and is safe and immunogenic in a Phase 1 trial	10+2 min
M3 🖵	**Stuart N. Isaacs** Philadelphia, PA, USA	Development of a next generation subunit smallpox vaccine using nucleoside-modified mRNA	10+2 min
M4 🧍	**Douglas W. Grosenbach** Corvallis, OR, USA	Smallpox Preparedness: Integrating TPOXX® into Response Planning	10+2 min
M5 🖵	**Gamal Wareth** Jena, DEU	WGS based analysis of antimicrobial resistance, MLST and motility in *Acinetobacter baumannii* in the One-Health context in Germany	10+2 min
M6 🧍	**Michael Hust** Braunschweig, DEU	Fighting SARS-CoV-2 and other infectious diseases with recombinant antibodies	10+2 min
M7 🧍	**Steve G. Lonsdale** Salisbury, GBR	Modernising Antibody Discovery for Biodefence (Dstl/CP133015)	10+2 min
M8 🧍	**Sebastian Jaksch** Garching, DEU	Design of a new model system for viral fusion	10+2 min
M9 🖵	**Scott A. Seitz** Boston, MA, USA	Advancement of a small molecule antiviral therapeutic to treat *Poxvirus* infection	10+2 min

M1

Vaccination against COVID-19 - the development of candidate vaccine MSARSCoV2

Gerd Sutter
Division of Virology, Department of Veterinary Sciences, LMU Munich, Munich, Germany

In a record time multiple safe and effective vaccines against COVID-19 have been developed. This talk will summarize recent progress obtained from the use of licensed SARS-CoV-2-specific vaccines and ongoing research as contribution to develop additional safe and efficacious vaccines against COVID-19. The Modified Vaccinia virus Ankara (MVA), a replication deficient and safety tested vaccinia virus, serves as vector technology platform to produce clinical grade recombinant MVA vaccines against emerging infections. Previously, a MVA vector vaccine against the Middle East respiratory syndrome coronavirus (MERS-CoV) proved safe and immunogenic in clinical use, providing an excellent blueprint for a candidate vaccine against the severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2). Here, we describe the generation, characterization and development of recombinant MVA viruses expressing full-length SARS-CoV-2 spike (S) proteins (MSARSCoV2). Preclinical vaccinations demonstrated solid MSARSCoV2 immunogenicity with the induction of S antigen-specific CD8+ T cells and virus neutralizing antibodies, and protective efficacy against SARS-CoV-2 challenge infections. MSARSCoV2 is currently being investigated in a phase Ib clinical trial.

M2

An MVA-based WEVEE vaccine protects mice against all 3 equine encephalitis viruses and is safe and immunogenic in a Phase 1 trial

Ariane Volkmann[1], Kathrin Endt[1], Robin Steigerwald[1], Jutta Müller[1], Darja Schmidt[1], Heinz

Weidenthaler[1], Lisa Henning[2], Jennifer Garver[2], and Elke Jordan[1]
1- Bavarian Nordic, Martinsried, Germany; 2- Battelle Memorial Institute, Columbus, USA

Equine encephalitis viruses (EEV) can cause severe disease of the central nervous system in humans, potentially leading to permanent damage or death. No licensed vaccine for human use is available to protect against these mosquito-borne pathogens, which can be aerosolized and therefore pose a bioterror threat in addition to the risk of natural outbreaks.

A multivalent alphavirus vaccine MVA-BN-WEV based on the modified vaccinia Ankara-Bavarian Nordic vaccine platform was generated that encodes the envelope polyproteins E3-E2-6K-E1 of all three EEVs. Immunogenicity and efficacy of a 2-dose regimen 4 weeks apart was evaluated in mice exposed to aerosolized EEV. An open-label, dose-escalating trial in healthy adults (N=45) evaluated safety and immunogenicity in subjects receiving the same vaccination regimen.

The majority of vaccinated mice exhibited VEEV, WEEV and EEEV neutralizing antibodies two weeks post second administration and all developed VEEV E2-specific T cell responses. Complete protection was demonstrated by the prevention of mortality and morbidity and lack of viremia in the homologous VEEV Trinidad Donkey challenge model, but also against heterologous VEEV INH-9813, WEEV Fleming and EEEV V105-00210 inhalational exposures.

In the Phase 1 trial, MVA-BN-WEV showed no clinically meaningful differences in the incidence of treatment-emergent adverse event (TEAE) between groups. Overall, 80.0% of all subjects experienced TEAEs during the active trial phase (unsolicited: 26.7%, general solicited: 55.6%, local solicited: 71.1%, mainly injection site pain 66.7%). Only one serious adverse event (pleural effusion; possibly related to study vaccine as assessed by the investigator) was reported by a subject in the lowest dose group nearly 3 weeks after the second vaccination. Robust VEEV E1 and E2-specific T cell responses and dose dependent VEEV-neutralizing antibodies were observed in all dose groups, with a durability of 6 months. A seroconversion rate of 93.3% was already achieved in the intermediate dose group.

In conclusion, MVA-BN-WEV encoding antigens of all three EEVs in a single vector was immunogenic and completely protective against all three EEVs in a mouse challenge model. Phase 1 data confirmed broad VEEV-specific immune responses (neutralizing antibodies and T cells) in humans and showed a favorable safety profile.

M3

Development of a next generation subunit smallpox vaccine using nucleoside-modified mRNA

Yuhong Xiao, Houping Ni, Jibin Zhou, Drew Weissman, and Stuart N. Isaacs
University of Pennsylvania, Medicine/Infectious Diseases, Philadelphia, PA, USA

Introduction: Although naturally occurring smallpox has been eradicated worldwide, a bioterrorism event or accidental release could result in re-emergence of this deadly disease. Also, ongoing human infections with monkeypox virus in central Africa could result in a virus that has enhanced human-to-human spread. Therefore, continued research into creating safer and effective smallpox vaccines is necessary given a non-immune population. Nucleoside-modified mRNA encapsulated in lipid nanoparticles (mRNA-LNP) has been demonstrated to elicit high and durable antibody responses to vaccine antigens and thus is a powerful vaccine platform. In this study, we intradermally immunized mice with mRNA-LNP encoding the poxvirus envelope protein L1 (L1-mRNA-LNP) and compared immune responses and protection to the protein-based subunit vaccine (L1/CpG/AH).

Material and Methods: L1 mRNA was made using T7 RNA polymerase on linearized plasmid encoding a truncated secreted L1-protein. The resulting purified mRNA contains a 5'-cap, a 101 nucleotide long poly(A) tail and incorporates 1-methylpseudouridine. The mRNA was encapsulated into LNPs. L1-mRNA-LNP (10 µg/mouse) were used to intradermally vaccinate 7-week old Balb/c mice. For the protein vaccination, purified baculovirus-expressed truncated L1 protein (2 µg/mouse) was formulated with Alhydrogel (100 µg/mouse) and CpG (50 µg/mouse) and intramuscularly injected into the gastrocnemius muscle. Eight weeks after the initial vaccinations, all mice were intranasally challenged with a lethal dose of vaccinia virus (VACV) and followed for weight loss.

Results: The groups of mice that received a single dose of either L1-mRNA-LNP or L1/CpG/AH induced similar antibody responses and virus neutralization antibody titers, but both groups lost significant weight after VACV challenge. Compared to the group of mice that was boosted with the subunit protein vaccine, the group of mice boosted with L1-mRNA-LNP generated significantly higher anti-L1 total IgG and IgG1 antibody response and virus neutralization antibody titers at all time points after boost. Also, while the protein-boosted mice lost significant weight, the L1-mRNA-LNP boosted group lost little to no weight indicating full protection

against a lethal VACV challenge.

Conclusion: These data demonstrate that L1-mRNA-LNP elicits high and protective immune responses and nucleoside-modified mRNA could become a platform for a future multi-subunit smallpox vaccine strategy.

M4

Smallpox Preparedness: Integrating TPOXX® into Response Planning

Douglas W. Grosenbach[1], William G. Burel[2], and Dennis E. Hruby[3]
1- SIGA Technologies, Inc., Poxvirus Research, Corvallis, OR, USA; 2- Hamilton Grace, President/Principal Consultant, Corvallis, OR, USA; 3- SIGA Technologies, Inc, Chief Scientific Officer, Corvallis, OR, USA

Smallpox is one of the most significant threats to health security and a single case anywhere worldwide would be considered a global health emergency. Although eradicated in nature, it still exists in secure laboratories in the US and Russia, and clandestine stores are thought to be held by other countries. Also, the virus can currently be synthesized *de novo* using modern synthetic biology technologies. Release into the population now would be devastating, as residual protective immunity has waned in the generation since routine vaccination ceased. Considering epidemiological and clinical features of smallpox, unchecked, it would rapidly become endemic, likely causing widespread panic, social upheaval, and disruption of commerce.

Most countries do not stockpile adequate smallpox countermeasures and would rely on international cooperation and access to smallpox vaccines and antiviral medicines held by WHO and donor countries, which would likely be insufficient based on lessons learned from the current COVID-19 pandemic. In 2018, TPOXX® (tecovirimat/ST-246) was approved in the US as the first smallpox antiviral for the treatment of smallpox disease and has been stockpiled in addition to enough ACAM2000® and JYNNEOS™smallpox vaccines to vaccinate all Americans.

In preparation for a smallpox outbreak, numerous independent models have been constructed to best inform response strategies. Although pre-event mass vaccination would most effectively prevent an outbreak, safety concerns with the vaccine preclude this approach. For outbreaks with a small number of index cases (10), ring vaccination strategies alone would likely be sufficient to control and eliminate the virus, while mass vaccinations would likely result in more deaths due to vaccination than from smallpox itself. In outbreaks with a higher number of index cases (≥1000), factors such as response times, efficiency of contact tracing, vaccination of suspected exposed contacts, and social cooperativity become critical factors. A suboptimal response would result in millions of lives lost and the return of endemic smallpox. No Smallpox Response Plan currently includes antiviral therapies such as TPOXX®, but inclusion in modeled scenarios dramatically improves outcomes. Smallpox Response Plans should be updated to include TPOXX® in addition to vaccination, and national stockpiles provisioned with adequate stores to respond most optimally to a smallpox outbreak.

M5

WGS based analysis of antimicrobial resistance, MLST and motility in *Acinetobacter baumannii* in the One-Health context in Germany

Gamal Wareth, Lisa D. Sprague, and Heinrich Neubauer
Friedrich-Loeffler-Institute, The Institute for Bacterial Infections and Zoonoses, Jena, Germany

Acinetobacter baumannii is a ubiquitous, Gram-negative bacterium associated with hospital-acquired infection globally. Multidrug resistant (MDR) strains were isolated from humans, farm and companion animals, wildlife species, foods, and the environment. Therefore, *A. baumannii* is considered a global One-Health problem with possible spread and transmission between these interconnected spheres. However, the full extent of the possible risk of human infection concerning animal contact, food consumption, and environmental contamination is unknown. Antibiotic susceptibility testing, motility and WGS analysis of 89 *A. baumannii* isolated from humans, animals and dried milk produced in Germany were investigated. Multilocus sequence typing (MLST) analysis based on Pasteur scheme identified 16 STs and seven new STs. ST/241 was the most prevalent in samples of non-human origin, whereas ST/2 was predominant in human samples. The highest resistant frequency was observed toward chloramphenicol (100%), followed by fosfomycin (96%) and cefotaxime (95%). The lowest resistant rates were observed toward colistin, trimethoprim/sulfamethoxazole, tigecycline and amikacin. A strain that harbored genetic resistance determinants was isolated from a horse with conjunctivitis. Antimicrobial resistance genes in sequenced strains and in the whole genome of 104 German isolates deposited in the NCBI database were investigated. The *ant*(3")-IIa confers resistance to aminoglycosides was the most

prevalent gene with a frequency of 100%, followed by the *bla* extsubscriptADC.25 confer resistance to cephalosporin with a frequency of 38.6%, and the two genes confer resistance to carbapenems, *bla* extsubscriptOXA-23 and *bla* extsubscriptOXA-51-like (*bla* extsubscriptOXA-66 variant), with a frequency of 29% and 26.5%, respectively. Around a quarter of genomes (26%) harbored *sul*2 that confer resistance to sulfonamides. The frequency of *tet*.B gene confer resistance to tetracycline was 19.5%, and the variants of acquired *bla* extsubscriptTEM were found with a frequency of 12%. *A. baumannii* exhibited different movement patterns, and strains containing *pil*A did grow faster. *A. baumannii* is a serious and emerging pathogen that causes nosocomial and community-acquired infections in Germany. The data on the impact of *A. baumannii* strains from (companion) animal origin on human health are still inconclusive. Further comprehensive one health genomic surveillance is needed to assess the possible zoonotic risk of this agent for human and animal health.

M6

Fighting SARS-CoV-2 and other infectious diseases with recombinant antibodies

Michael Hust
TU Braunschweig, Biotechnologie, Braunschweig, Germany

Antibody phage display is a key *in vitro* technology to generate human antibodies against pathogens and toxins. In this presentation, our pipeline for the development of neutralizing human and human-like antibodies using naive and immune/patient derived antibody gene libraries will be outlined. Examples of our pipeline will be given for the development in vivo protective antibodies against alphaviruses, Marburg virus, Ebola Sudan virus and SARS-CoV-2 as well as for botulinum toxins A, B and E (EU AntibotABE project) and diphtheria toxin (DATMAB project). These antibodies are potential lead candidates for further clinical and regulatory development.

M7

Modernising Antibody Discovery for Biodefence (Dstl/CP133015)

Grace M. Howells, Claire L. Lonsdale, and Steve G. Lonsdale
Dstl, CB Sense & Protect, Salisbury, United Kingdom

Since Köhler and Milstein's seminal work on hybridoma generation, monoclonal antibodies have revolutionised life science research as well as providing critical capability in the Biodefence arena as therapeutic agents, diagnostic reagents and environmental detection reagents. However, there are limitations to the technology: most notably that the efficiency of hybridoma generation is very low. This facet often results in expensive and time-consuming discovery projects.

The UK Ministry of Defence supports a productive hybridoma generation capability at the Defence Science and Technology Laboratory (Dstl), Porton Down and also carries out research to drive improvements to the efficiency and sustainability of antibody discovery for Biodefence.

In this presentation, I shall describe an ongoing project to utilise high throughput sequencing of native-pairing antibody gene transcripts from single cells for the purpose of antibody discovery. I shall describe the use of model antigens such as *Coxiella burnetii* and *Clostridium botulinum* neurotoxin type A to characterise the process and demonstrate a pathway to efficient verification of the sequencing output. I shall show that, even in its most basic format, this approach constitutes substantially enhanced interrogation of a host's antibody response when compared to hybridoma generation.

M8

Design of a new model system for viral fusion

Dominic Hayward[1], Henrich Frielinghaus[1], Olaf Holderer[1], Rosina Ehmann[2], Joachim Bugert[2], and Sebastian Jaksch[1]
1- Forschungszentrum Jülich, Jülich Centre for Neutron Science (JCNS) at Heinz Maier-Leibnitz Zentrum (MLZ), Garching, Germany; 2- Bundeswehr Institute of Microbiology, Munich, Germany

The fusion of viral and host cell membranes is a pivotal step in the infection and life cycle of any virus. The *coronaviridae* present a taxonomic family with a complex and highly diverse fusion behavior across different species and viral host environments. Despite the massive global research interest in SARS-CoV-2 many aspects of the fusion process are still only rudimentarily understood. Biological fusion assays are widely applied to study

different steps of viral-host membrane fusion, however, multidisciplinary approaches offer a broader range of parameters to study. Here, we report the establishment of a new model system for viral fusion based on the neutron scattering behavior of tailored unilamellar lipid vesicles with specific membrane proteins.

Our target was to design individual vesicles from cellular material which only contain the membrane proteins included in the initial cellular plasma membrane and none of the organelle membranes within the cell. Thus, by protein expression on the cells, individual virion and target vesicles could be designed. We were able to ascertain that ultrasonic cell disruption delivers robustly reproducible cellular debris, which by differential centrifugation can be cleared of virtually any non-membrane proteins and organelle debris. The results of creating 100 nm unilamellar vesicles by extrusion were confirmed by several methods, among the dynamic light scattering as well as small-angle X-ray and neutron scattering.

In order to investigate specific features of infection by vesicle fusion in the initial infection stages of SARS-CoV-2 this model system can be fitted with any viral or host cell membrane protein on the surface. In addition, first deuteration studies were carried out. Using this system, we are now able to assess the impact of coronavirus spike, ACE2 or other (proteolytic) membrane proteins on vesicle fusion, as well as investigate the impact of soluble compounds like drugs or pH modifiers. Using deuterated samples and neutron scattering gives us access to timescales in the range of milliseconds, adding valuable information about the very early fusion time regime.

M9

Advancement of a small molecule antiviral therapeutic to treat *Poxvirus* infection

Scott A. Seitz[1], Paul Marcyk[2], Andrew De Los Santos[2], Lauren E. Brown[2], Scott E. Schaus[2], and John H. Connor[1]
1- Boston University-NEIDL, Microbiology, Boston, MA, USA; 2- Boston University, Chemistry, Boston, USA

Poxviruses are a causative agent of a broad range of human diseases responsible for countless mortalities and represent a large economic burden worldwide. Although Smallpox eradication through vaccination has decreased the global burden of the Poxvirus family, ongoing human infections (such as Monkeypox) and the potential use of *Variola* as a bioterrorist agent facilitates the need for the development of effective therapeutics. We developed a class of small molecule compounds and have described their broad efficacy against the Poxvirus family based off a drug designated CMLDBU6128 which shows strong viral inhibition in micromolar concentrations. Utilizing this chemical structure, we made various chemical modifications and tested several against *Vaccinia* virus, a prototypical Poxvirus. We found several molecules that improve *Vaccinia* inhibition in comparison to the parent 6128 compound, with some showing activity in the nanomolar concentration with minimal cytotoxicity. We show the mechanism of action is post viral entry using a reporter virus that expresses GFP in the late stage of infection. These compounds represent a new class of inhibitors that could be developed and utilized as antiviral measures against Poxviruses.

Garden Hall / 16:00 ... 18:00

N

International biosecurity

Chairs: Erik Tintrup (DEU) and Gordon Wilke (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
N1 🧍	**Erik Tintrup** Berlin, DEU	The Federal Foreign Office's engagement in Biosafety and Biosecurity: Reducing global biological threats in a collaborative effort	12+3 min
N2 🧍	**Bourema Kouriba** Bamako, MLI	Role of the mobile laboratory in the response to the COVID-19 pandemic in the region of Timbuktu, Mali	12+3 min
N3 🖵	**Mohamed Ben Moussa** Tunis, TUN	The Tunisian Rapid Response Team and Mobile Laboratory – From Training and Field Exercises to Providing Essential Diagnostic Support for Tunisia during the COVID-19 pandemic	12+3 min
N4 🧍	**Lela Bakanidze** Tbilisi, GEO	Awareness Raising Campaign in the Framework of EU Chemical, Biological, Radiological and Nuclear (CBRN) Centers of Excellence (CoE) Initiative in Central Asia	12+3 min
N5 🖵	**Eva Mertens** Hamburg, DEU	Introducing the '*Global Partnership Initiated Biosecurity Academia for Controlling Health Threats (GIBACHT) – COVID-19 Strategy (COST-19)*' Initiative to strengthen the SARS-CoV-2 response in partner countries	12+3 min
N6 🧍	**Nur Tukhanova** Munich, DEU	Tula Orthohantavirus (TULV) as the predominant species in three regions in Kazakhstan	12+3 min
N7 🧍	**Anna Shin** Almaty, KAZ	Characterization of TBEV in Kazakhstan by serological and molecular biological methods	12+3 min
N8 🧍	**Maryna Y.U. Kit** Kharkiv, UKR	Oral fluid-based surveillance study of African Swine Fever amoung wild boars in Ukraine	12+3 min

N1

The Federal Foreign Office's engagement in Biosafety and Biosecurity: Reducing global biological threats in a collaborative effort

Erik Tintrup
Federal Foreign Office, Deputy Head of the Division for Biological and Chemical Weapons Issues and Director of the German Biosecurity Programme, Berlin, Germany

The SARS-CoV-2-Crisis showcased how vulnerable countries are to biological threats. Dangerous pathogens do not respect borders. Hence, the deliberate misuse of highly pathogenic agents is a risk not only for individual countries, but for entire regions. Proliferation is fuelled by severe conflicts, international terrorism, global trade and growing mobility as well as dual use research and technological development in the life sciences. In order to reduce these risks on a global scale, cross-border cooperation in the spirit of partnership is needed.

In this context and as part of Germany's preventive security policy, the Federal Foreign Office (FFO) advances partner countries' capacities through the German Biosecurity Programme, initiated in 2013, and biosecurity projects within the Federal Government's Enable and Enhance Initiative, launched in 2016. All these efforts contribute to the overarching goal of strengthening the Biological Weapons Convention, which, in August 2022, will conduct its very important 9th Review Conference.

The Biosecurity Programme is currently in its third phase. It is active in nine partner countries and two supraregional projects. All projects are implemented in cooperation with five leading German institutions. The conducted activities focus on training measures in the areas of biosafety and biosecurity, surveillance, detection and diagnostics, awareness rais-

ing, networking and capacity development. Hereby, the programme adds to the G7 Global Partnership against the Spread of Weapons and Materials of Mass Destruction, which Germany will be chairing in 2022. The Programme is intended to support partner countries tackle biological threats, such as potential bioterrorist attacks and other ways of deliberate misuse of dangerous pathogens and toxins or outbreaks of highly pathogenic diseases and pandemics.

The efforts within the Enable and Enhance Initiative aim at strengthening national security capacities and enabling partners to react to regional biological threats. Project activities are implemented in seven partner countries. The initiative is coordinated by the FFO and the Federal Ministry of Defence.

Recently, the pandemic had a big impact on the Biosecurity projects. The established structures and enhanced capabilities were put to the test and trusting co-operations as well as the flexibility of all actors supported the pandemic response of the partner countries. Thus, the projects have shown strength and enormous adaptability.

N2

Role of the mobile laboratory in the response to the COVID-19 pandemic in the region of Timbuktu, Mali

Bourema Kouriba[1], Abdoul karim Sangare[1], Bréhima Traore[1], Elisabeth Sogodogo[1], Judicael Ouedraogo[1], Abderrhamane Maiga[1], Zoumana Doumbia[2], Charles Dara[3], and Djibril Kassogué[3]
1- Centre d'Infectiologie Charles Merieux-Mali, Bamako, Mali; 2- Regional Health Department of Timbuktu, Timbuktu, Mali; 3- Timbuktu Hospital , Timbuktu, Mali

The COVID-19 pandemic officially affected Mali on March 25, 2020. The epidemic rapidly evolved with intense community transmission with diagnostic difficulties which was provided by only 4 laboratories all located in Bamako, the capital city of Mali. It is in this context that the epidemic particularly flared in the city of Timbuktu located in northern Mali more than 1000km from Bamako. To respond to this outbreak, the Ministry of Health of Mali had requested the deployment of the mobile laboratory in Timbuktu to allow on-site diagnosis. From May 29 to July 6, 2020, the intervention team of CICM, deployed the mobile laboratory in Timbuktu. The tests were carried out and the results transmitted within 4 hours after receiving the samples. Patients with a positive test were systematically placed in isolation and treated in the center. This strategy of "testing and isolating the positives" with contact tracing has significantly reduced contamination and mortality. In fact, before the deployment of the mobile laboratory, the samples were sent to Bamako by flight by UNHAS (United Nation Humanitarian Air Service) or MINUSMA (United Nations Multidimensional Integrated Stabilization Mission in Mali) twice a week. The results were returned 72 hours later, with some omissions either due to data processing difficulties or loss of samples. There had been 8 deaths due to respiratory distress in a week at the Timbuktu Hospital, which was destabilizing for the authorities and the population of the city located in an insecure zone. The lack of an on-site diagnostics caused a delay in the management of patients infected with COVID-19. With the deployment of the mobile laboratory, the diagnostic being early, mortality decreased significantly. The dynamics of the outbreak have changed with a significant decrease of contamination, hospitalization, and death from COVID-19 in Timbuktu during the first wave in Mali. A total of 1516 people were tested, of which 440 were positive, for a prevalence of 29%. Patients were isolated either in the hospital treatment center or in their homes. Only 3 deaths were recorded during the deployment period which lasted 43 days. This deployment of the mobile laboratory in an epidemic situation was an opportunity to test the competence of the CICM-Mali team after several training sessions by experts from the Institute of Microbiology of Bundeswehr (IMB) as part of the German Ministry of Foreign Affairs' "Enable and Enhance Initiatives project".

N3

The Tunisian Rapid Response Team and Mobile Laboratory - From Training and Field Exercises to Providing Essential Diagnostic Support for Tunisia during the COVID-19 pandemic

Mohamed Ben Moussa[1], Sameh Khairallah[1], Achref Ben salah[1], Lobna Sebei[1], Susann Zschornack[2], Stefanie Bauer[2], Simone Eckstein[2], Kilian Stoecker[2], and Roman Woelfel[2]
1- l'Hôpital Militaire Principale de Tunis, Département de Virologie, Tunis, Tunisia; 2- Bundeswehr Institute of Microbiology, Munich, Germany

A key part of the German government's Enable and Enhance Initiative in Tunisia was the delivery of a mobile laboratory in 2017 and the extensive training of a Tunisian rapid response team in the same and the following years.

During a first field training in Tunisia, the German trainer team simulated an outbreak of the fictional new-emerging human pathogen MS2 in Tunisia to test the diagnostic accuracy under real-life condi-

tions. During this one-week training, the team analyzed samples of various matrices, like blood, urine, oral swabs, feces, breast milk or semen with an accuracy rate of 97.8 %. In a second field exercise in 2019, the Tunisian mobile laboratory was deployed to Burkina Faso to assist during a simulated outbreak of a new hemorrhagic fever in the context of the Enable and Enhance Initiative in the G5-Sahel region. During these trainings, the rapid response team evaluated and optimized several critical points regarding the usage of the mobile laboratory equipment, as well as the human resources needed to run the molecular diagnostics.

In 2019, a new human pathogen called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the disease COVID-19, emerged. Since the methods used in the diagnostic of the fictional pathogens could be easily adapted for the diagnostic of SARS-CoV-2, we had already implemented standardized procedures for SARS-CoV-2 diagnosis at the Military Hospital of Tunis and in the mobile laboratory in February 2020. In spring 2020, when the first SARS-CoV-2 infections were officially confirmed in Tunisia, the mobile laboratory was immediately deployed to regions in Tunisia that did not have laboratories for the diagnosis of SARS-CoV-2. The scope of these missions was to support the national effort in the diagnosis of SARS-CoV-2 and to bring the diagnosis closer to the scene of the epidemic. Since then, the rapid response team has successfully carried out ten missions in various remote regions in Tunisia: three times to Kebili and once to Sfax, Siliana, Tataouine, Gabes, Sidi Bouzid, Kasserine, and Kairouan, respectively. During these missions, two teams of four persons operated the mobile laboratory in two shifts and analyzed over 300 patient samples per day, with a total analysis number of over 20.000 patient samples up to today. This makes the mobile laboratory and the rapid response team a vital pillar in the management of the COVID-19 pandemic by the Tunisian Ministry of Health.

N4

Awareness Raising Campaign in the Framework of EU Chemical, Biological, Radiological and Nuclear (CBRN) Centers of Excellence (CoE) Initiative in Central Asia

Bakhtior Gulyamov[1], Lela Bakanidze[2], Iryna Yarema[1], Otabek Kasimov[1], and Jasurjon Ibragimov[1]
1- EU CBRN CoE RS for CA, Tashkent, Uzbekistan; 2- EU CBRN CoE RS for CA, Tbilisi, Georgia

The European Union (EU) Chemical, Biological, Radiological and Nuclear (CBRN) Centers of Excellence (CoE) Initiative aims to strengthen the institutional capacity of countries outside Europe to mitigate CBRN risks, coming from criminal activities, natural disasters or accidental releases. The objective of the CoE Initiative is to develop a structural, all-hazards CBRN policy at the domestic, regional and international levels to anticipate and respond to these risks, and to reduce the vulnerability of countries to CBRN events. The initiative is in the reciprocal interests of regional and EU security. The approach adopted by the EU in establishing the CBRN CoE Initiative is innovative and broad-ranging. It aims to provide assistance in the implementation of international commitments to mitigate CBRN proliferation risks.

Security measures and seismic conditions in Central Asia, as well as strategic importance within the former USSR, strongly reflect the presence of natural, man-made and formerly militarized CBRN threats. Much of the mentioned infrastructure was dismantled before or after the dissolution of the Soviet Union, the region remains to be CBRN hazard-prone. The fact that Central Asian countries are vulnerable to natural disasters (earthquakes, landslides, floods, etc.) exacerbates CBRN risks.At the same time, the region's multimillion population should be aware of these CBRN threats.

The innovative project "Popularization and Visibility of the EU CBRN CoE Initiative among the Various Layers of the Population" seeks to raise the awareness of CBRN issues among children and youth through organization of lessons, meetings, etc. Within the frame of the project, trainings for schoolchildren and university students in Tashkent, Karakalpakstan, Namangan, Samarkand, Khorezm, Bukhara and Tashkent regions of Uzbekistan were conducted. The training materials varied depending on the age group and specialization of the audience.

The EU CBRN CoE Regional Secretariat plans to expand the geography of these trainings and share the experience with the neighbouring regions as well as increase the scope of the campaign in Central Asia, especially by covering aspects on biosafety, bioeconomics, CBRN security culture, etc.

N5

Introducing the "*Global Partnership Initiated Biosecurity Academia for Controlling Health Threats (GIBACHT) - COVID-19 Strategy (COST-19)*" Initiative to strengthen the SARS-CoV-2 response in partner countries

Eva Mertens[1], Lea C. Wende[2], Janine Dywicki[2], Axel Hoffmann[3], Barbara M. Bürkin[3], Joachim Pelikan[3], and Elizeus Rutebemberwa[4]

1- Bernhard Nocht Institute for Tropical Medicine, Infectious Disease Epidemiology, Hamburg, Germany; 2- Robert Koch Institute, ZIG3 - Centre for International Health, Preparedness and Operations Support, Berlin, Germany; 3- Swiss Tropical and Public Health Institute, Education & Training, Teaching Technologies & Didactics, Basel, Switzerland; 4- African Field Epidemiology Network, Kampala, Uganda

The Global Partnership Initiated Biosecurity Academia for Controlling Health Threats (GIBACHT) is a multilateral one-year biosafety and biosecurity training programme, that is currently running its seventh cohort. GIBACHT is responding to the need of countries with insufficiently prepared health systems and risk of accidental and deliberate release of infectious agents.

The programme is supported by the German Biosecurity Programme, coordinated by the Bernhard Nocht Institute for Tropical Medicine (BNITM) and conducted in partnership with the Robert Koch Institute (RKI), the Swiss Tropical and Public Health Institute (Swiss TPH), and the African Field Epidemiology Network (AFENET). GIBACHT reaches specialists from public health institutions in partner countries in Africa, the Middle East, and South and Central Asia, who play a key role in managing biological incidents.

In early 2020, a survey among the GIBACHT alumni network revealed that many of them are engaged in multiple stages of the COVID-19 pandemic response and that capacity building and training opportunities including practical exercises in this field are highly needed.

The GIBACHT consortium was granted an additional budget by the German Biosecurity Programme to award funding to COVID-19 microprojects developed by GIBACHT alumni aimed at the containment, control and prevention of ongoing or future COVID-19 outbreaks in their countries. This initiative, called "(GIBACHT) - COVID-19 Strategy (COST-19)", offered support for three types of training measures: i) digital trainings in form of an online workshop, ii) face-to-face simulation exercises providing in-depth training for health care workers and technical staff directly involved in COVID-19 outbreak prevention and control, and iii) information campaigns to improve knowledge regarding COVID-19 among the general public. Out of 28 submitted proposals, six projects, which will herein be presented, were selected and successfully implemented by the GIBACHT alumni.

The COST-19 Initiative aims at strengthening both international and national networks in target countries, thereby empowering sustainable structures for continuing education in infectious disease epidemiology, biosecurity, and disaster risk reduction.

N6

Tula Orthohantavirus (TULV) as the predominant species in three regions in Kazakhstan

Nur Turkhanova[1,2], Anna Shin[1,2], Nurkeldi Turebekov[2], T. Nurmakhanov[2], A. Shevtsov[3], Karlygash Abdiyeva[4], V. Sutyagin[5], Almas Berdibekov[5], Nurkbek Maikanov[5], Aleksey Adruyhenko[5], Lyazzat Yeraliyeva[6], Guenther Froeschl[1,7], Michael Hoelscher[7], Edith Wagner[8], Lukas Peintner[8], and Sandra Essbauer[8]

1- Center for International Health, University Hospital, LMU, Munich, Germany; 2- Central Reference Laboratory, NSCEDI, Almaty, Kazakhstan; 3- National Center for Biotechnology, Nursultan, Kazakhstan; 4- Branch National Center for Biotechnology, Almaty, Kazakhstan; 5- NSCEDI Antiplague Station, Kazakhstan; 6- National Scientific Center of Phthisiopulmonology, Almaty, Kazakhstan; 7- Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU, Munich, Germany; 8- Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, Munich, Germany

Orthohantaviruses (OHV) are zoonotic pathogens that play a significant role in public health. Several small mammals are reservoirs of OHV and can cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) in humans. The first human case of HFRS was registered in West Kazakhstan region in the year 2000. A following investigation into small mammals in West Kazakhstan showed antigens for OHV in wild living rodents in four districts of West Kazakhstan region and proved a natural reservoir for OHV. So far, molecular genetic data on OHV in Kazakhstan are limited. The aim of this study is to investigate small mammals for the presence OHV by molecular methods and to provide a molecular characterization of the circulating strains in Kazakhstan.

The study was set up in 2018-19. Small mammals were trapped in West Kazakhstan region, Almaty region and Almaty city throughout the year.

In total 621 small mammals from 11 species were investigated. Among the collected small mammals 2.4% tested positive for orthohantavirus S segment. The prevalence of positive samples for OHV was 0.4% in West Kazakhstan and 7% in Almaty region respectively. Sequencing parts of the S segment specified Tula OHV (TULV) as the predominant virus in these two regions.

Our data showed that geographical distribution

of TULV OHV is more extended as previously thought. Two species of rodents (*Microtus arvalis* and *Dryomys nitedula*) were positive for TULV in Kazakhstan. It needs further investigation of host reservoirs among the country to understand the full distribution of OHV in Kazakhstan.

N7

Characterization of TBEV in Kazakhstan by serological and molecular biological methods

Anna Shin
NSCDEI, Virology, Almaty, Kazakhstan

Flaviviruses are the cause of many diseases in humans. Their similarity in the antigenic structure causes a cross-reaction that complicates the diagnostic of infections. Tick-borne encephalitis virus (TBEV), a member of the flavivirus family, is the cause of tick-borne encephalitis (TBE). In Kazakhstan (KAZ), 363 cases of TBE were registered in 2011-2020. However, there is a lack of flavivirus serological investigation in humans as well as limited genomic data such as TBEV E-genes and complete genomes for the territory of KAZ. Here, we want to determine the TBEV subtypes that circulate in KAZ, and investigate on the prevalence of antibodies against TBEV in patients with suspected meningitis.

More than 10,000 ticks and serum with cerebrospinal fluid (CSF) from 179 patients with suspected cases of meningitis were collected in three endemic regions of KAZ (East Kazakhstan Oblast (EKO), Almaty (AO) and Akmola Oblast (AkO)). After sorting ticks into pools by sex and species and following homogenization, tick pools were tested for TBEV. Subsequently, an E-gene sequencing was carried out. The human samples were tested with ELISA, IIFT, and real-time RT-PCR for infections with flaviviruses.

TBEV RNA was detected in eight pools of EKO, in two pools of AkO and in two pools of AO in isolates from *Ixodes persulcatus* and in *Dermacentor marginatus*. All TBEV samples belong to the Siberian subtype closely related to strains from China and Western Siberia when collected in EKO or related to strains from Western Siberia when from AO. By analyzing the human sera, we surprisingly found next to TBEV also other flaviviruses such as WNFV and OHFV.

In this study, we were able to draw a more comprehensive picture of TBEV distribution and heritage in three areas of KAZ. In addition, our data showed that OHFV and WNFV play in addition to TBEV a role in patients with undifferentiated meningitis.

N8

Oral fluid-based surveillance study of African Swine Fever amoung wild boars in Ukraine

Maryna Y.U. Kit[1], Claudia Popp[2], Heiner von Buttlar[2], Anton P. Gerilovych[1], and Julia Schwarz[2]
1- National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Department for molecular epidemiology and diagnostics , Kharkiv, Ukraine; 2- Bundeswehr Institute of Microbiology, Department of Bacteriology and Toxicology, Munich, Germany

African swine fever (ASF) is a contagious disease of domestic pigs and wild boar caused by African swine fever virus (ASFV). An acute form of the disease results in up to 100% mortality rate. However, when the disease becomes endemic on new territory, the clinical symptoms become milder and the mortality rate decreases. Survivor animals form antibodies and can spread the virus in a population (Eble et al., 2019).

More than 540 cases of ASF have been registered in Ukraine since the first outbreak in 2012. Since 2018, despite any additional biosecurity measures the number of registered disease cases decreases constantly (2018 - 145 cases, 2019 - 53 cases, 2020 - 28 cases, 2021 - 6 cases). This might be a marker for ASF endemicity in Ukraine. We assume that a hidden spread of the ASFV might occur in the wild boar population including survivor animals.

Therefore, the aim of our work is a surveillance study of ASF in the wild boar population in Ukraine. Oral fluid could be an alternative type of clinical material for diagnostics since the sampling process is non-invasive, animal-friendly and stress-free. Oral fluid contains IgA, IgM and IgG antibodies and a variety of infectious agents, so it could be tested with both molecular and serological methods (Pricket and Zimmerman, 2010).

Thus, we collected oral fluid from wild animals using a rope-in-a-bait sampling technique (Mouchanat et al., 2014). For this purpose, we used ropes embedded in a matrix consisting of cornmeal, milk powder, coconut oil, paraffin wax and almond aroma. The baits have been distributed in forests in Kharkiv oblast (Ukraine) in 2020 and 2021 during winter when food supply was limited for animals. The study locations are known to be inhabited with wild boars. The exposure time varied from 7 to 21 days for different baits.

Totally we distributed 240 baits and collected 103 samples of ropes chewed by wild animals. To obtain oral fluid, we incubated chewed ropes with 5 ml of saline buffer for 30 minutes at 25 extsuperscriptoC.

After the incubation, we placed the ropes in syringes and squeezed the liquid into plastic tubes.

To ensure the oral fluids collected belong to wild boars we will perform detection of *Sus scrofa COX1* gene by PCR. All the *Sus scrofa* positive samples will be tested for ASFV *c962r* gene presence using loop-mediated isothermal amplification (unpublished). Furthermore, we will test the samples for presence of antibodies against ASFV by newly developed ELISA.

Audimax / 08:30 ... 10:10

O Bacteriophages against highly pathogenic and MDR bacteria: from diagnostics to therapy (Part 1 of 2)

Chairs: Peter Braun (DEU) and Samuel Kilcher (CHE)

Session summary

Presentations are online (▭) or on-site (👤) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
O1 👤	**Samuel N. Kilcher** Zurich, CHE	Reporter Phage-Based Detection of Bacterial Pathogens: Design, Engineering, and Application	25+5 min
O2 👤	**Jiemin Du** Zurich, CHE	Rapid Detection of Bacterial UTIs via Reporter Phage-Based Bioluminescence Assay	12+3 min
O3 👤	**Peter Braun** Munich, DEU	Recombinant receptor binding proteins of bacteriophages as versatile tools for pathogen detection	20+5 min
O4 👤	**Nadja Rupprich** Munich, DEU	Enzyme-linked phage receptor binding protein assay (ELPRA) enables identification of *Bacillus anthracis*	12+3 min
O5 👤	**Andrew Millard** Leicester, GBR	Arming bacteriophages with colicins for more effective killing	12+3 min

O1

Reporter Phage-Based Detection of Bacterial Pathogens: Design, Engineering, and Application

Samuel N. Kilcher
ETH Zurich - Institute of Food, Nutrition, and Health, D-HEST, Zurich, Switzerland

Reporter bacteriophages (phages) are genetically engineered to deliver a reporter gene that is expressed within infected bacteria and subsequently detected to indicate the presence of viable host cells. Due to the rapid kinetics and high specificity of phage infection, reporter phages enable fast and reliable pathogen surveillance and detection in clinical samples, contaminated food products, and within production and processing sites. The development of reporter phages hinges on the identification of phage candidates with suitable host-range and on the availability of efficient genome engineering tools to insert reporter genes. In this presentation, I will discuss recent advances in phage engineering technology with a focus on synthetic biology and CRISPR-Cas9-assisted platforms. These novel tools are applicable to many bacterial genera and can be used to adapt phage host-specificity and to rapidly construct and optimize reporter phage scaffolds. Besides facilitating pathogen identification and differentiation, these platforms can also support personalized phage therapy by providing a rapid companion diagnostic tool to determine the susceptibility of patient isolates to therapeutic phage products.

O2

Rapid Detection of Bacterial UTIs via Reporter Phage-Based Bioluminescence Assay

Jiemin Du, Susanne Meile, Martin J. Loessner, Samuel N. Kilcher, and Matthew Dunne
ETH Zurich - Institute of Food, Nutrition, and Health, D-HEST, Zurich, Switzerland

Bacterial urinary tract infections (UTIs) are among the most common microbial diseases in all age groups and major contributors to the injudicious use of antibiotics. Rapid and accurate pathogen identification directly from urine samples can drastically improve the clinical management of UTIs, alleviating their socio-economic impacts and facilitating antibiotic stewardship. Luciferase reporter phages are genetically engineered viruses capable of rapidly detecting their target bacterial hosts with exquisite specificity and sensitivity. As signal production requires phage genome injection and subsequent host-dependent expression of phage-encoded reporter proteins, this diagnostic method does not produce false-positive results in the presence of dead target cells. Here, using a set of six genetically distinct luciferase reporter phages, we report the development and evaluation of a novel phage-based bioluminescence assay for rapid detection of the three predominant UTI pathogens, *E. coli*, *Klebsiella spp.* and *Enterococcus spp.* *In vitro*,

with pure cultures, the assay positively identified 89% of 155 relevant clinical isolates, showing the highest inclusivity for *Enterococcus spp.* (50/52), followed by *Klebsiella spp.* (45/51) and *E. coli* (43/52). Cross-reactivity of the phage cocktails with other closely related genus including *Raoultella* and *Staphylococcus* was observed, but only at minimal rate (4.3%; 3/70). The assay performance was further assessed alongside routine clinical testing on 145 patient specimens collected from a local hospital in Switzerland over 5 months. Following short pre-enrichment steps and with simple substrate addition, the assay reliably detected the three target pathogens with 73% sensitivity and 96% specificity at a resolution of $\geq 10^3$ CFU/ml in less than 6 h. This phage-based diagnostic platform offers opportunities for prompt bacterial UTI diagnosis in point-of-care settings and could be harnessed as companion diagnostics to assist rapid identification of potential responder patients in the course of a phage therapy.

O3

Recombinant receptor binding proteins of bacteriophages as versatile tools for pathogen detection

Peter Braun, Franziska Dähner, Diana Neif, Linda Dobrzykowski, and Gregor Grass
Bundeswehr Institute of Microbiology, Munich, Germany

For highly pathogenic bacteria, such as *Yersina pesits* or *Bacillus anthracis*, rapid and unambiguous detection is crucial for timely antibiotic therapy of infected patients. Polymerase chain reaction (PCR) is the gold standard for diagnostics of most infectious diseases. While detection of specific genes by PCR can be achieved within a few hours, the presence of intact bacteria can only be verified after one to several days usually by culture based methods. For decades, plaque assays using highly specific bacteriophages have been widely used as low-tech alternatives to PCR. However, phage susceptibility assays are time consuming and typically require pure cultures of suspect isolates. Using phage receptor binding proteins (RBPs) instead of whole phages can facilitate and accelerate phage-based pathogen detection. RBPs, which may be, e.g. phage tail-fibers or -spikes, are employed by the virus to recognize specific surface structures on bacterial host cells and therefore typically determine phage specificity. Recombinant RBPs can be produced as genetic fusions with reporter proteins, such as enzymes or fluorescent proteins, and thus used as molecular probes for pathogen detection. In addition, RBPs may be coupled to magnetic beads and serve as highly specific capture molecules for bacterial enrichment or isolation approaches. Here, we produced putative RBPs of various phages specific for *B. anthracis* or *Y. pestis* as fusions with fluorescent proteins. From this, we developed microscopy-based approaches for the facile, rapid and highly specific detection of these notorious pathogens in pure cultures and in clinically relevant matrices.

O4

Enzyme-linked phage receptor binding protein assay (ELPRA) enables identification of *Bacillus anthracis*

Nadja Rupprich, Peter Braun, Diana Neif, and Gregor Grass
Bundeswehr Institute of Microbiology, Munich, Germany

The notorious zoonotic disease anthrax, caused by the spore forming bacterium *Bacillus anthracis*, is typically identified by molecular methods such as polymerase chain reaction (PCR). Due to the close relationship of *B. anthracis* with other species of the *Bacillus cereus sensu lato* group, differentiation of the pathogen from its close relatives is challenging by other means especially when working with environmental samples. Due to lack of unique morpholocial traits of *B. anthracis* cells and colonies, screening of suspect colonies from enrichment agar plates can be time consuming.

Here, we designed and evaluated new tools for the rapid detection of *B. anthracis* colonies using two implemenations of enzyme-linked phage receptor binding protein assays (ELPRA). Receptor binding proteins (RBPs) are typically phage tail-fibers or -spikes, and are required by the virus to recognize specific surface structures on its bacterial host cells. Employing horseradish peroxidase (HRP) coupled RBPs we developed a single-tube centrifugation assay simplifying the rapid analysis of suspect colonies within a few minutes. In a second assay, a RBP fusion with nanoluciferase (NLuc) facilitated the identification of suspect colonies from mixed overgrown solid (agar) media derived from the complex matrix soil. Both tests identify vegetative cells of *B. anthracis* with little processing time and may support or confirm pathogen detection by molecular methods such as PCR.

O5

Arming bacteriophages with colicins for more effective killing

Christian Harrison[1], Martha Clokie[2], Nick

Waterfield[3], and Andrew Millard[2]
1- University of Leicester, Genetics and Genome Biology, Leicester, United Kingdom; 2- University of Leicester, Genetics and Genome Biology, Leicester, United Kingdom; 3- University of Warwick, Warwick Medical School, Coventry, United Kingdom

Bacteriophages are natural efficient killers of their bacterial hosts and offer promise as therapeutic agents, as the number of antibiotic-resistant bacterial infections increases. The specific nature of bacteriophages and their self-replicating nature makes them attractive as therapeutic agents. While there any many benefits of utilising phages, they do have limitations. These limitations include their host specificity, which means all strains of bacteria won't be killed by a single phage. In addition over time their bacterial hosts can evolve resistance. Some of the limitations can be overcome by the careful selection of phages and combing to form phage-cocktails.Alternatively, phages can be engineered to expand the range of bacteria they can kill.

Here we have engineered a T4like phage to carry different colicins. Colicins are small molecules produced by *Escherichia coli*, which are toxic to other *E. coli* that do not have an immunity protein to provide protection against that colin. We have assessed the effect of engineering a coliphage to produce colicins during the infection process. We have established that colicin genes can be stably maintained in the genome of a coliphage. These colicin-containing phages are more efficient in killing their bacterial hosts. In addition, these phages are able to kill previously phage-resistant phage *E. coli* mutants and a wider range of *E. coli* when co-cultured with multiple *E.coli* strains.

Audimax / 10:30 ... 12:30

P Bacteriophages against highly pathogenic and MDR bacteria: from diagnostics to therapy (Part 2 of 2)

Chairs: Christian Willy (DEU) and Joachim J. Bugert (DEU)

Session summary

Presentations are online (🖵) or on-site (🧍) as marked in the TalkID column. Order may be changed.

TalkID	Speaker	Title	Duration
P1 🖵	**Martha Clokie** Leicester, GBR	Phage Therapy in Europe	20+5 min
P2 🖵	**Alla Yushchenko** Kyiv, UKR	Initial host range analysis of commercial phage preparations on Ukrainian MRSA isolates	12+3 min
P3 🖵	**Alexander Harms** Basel, CHE	Learning from bacteriophages how to overcome the resilience of antibiotic-tolerant, dormant bacteria	12+3 min
P4 🧍	**Simone Eckstein** Munich, DEU	Isolation, characterization and engineering of lytic bacteriophage TUN1 specific for *Klebsiella pneumoniae* K64 clinical isolates from Tunisia	12+3 min
P5 🖵	**Holger Ziehr** Braunschweig, DEU	Bacteriophages – Old antiinfectives with new perspectives	12+3 min
P6 🧍	**Kilian Vogele** Garching, DEU	A cell-free platform for production, modification and engineering of bacteriophages for therapeutic use	12+3 min
P7 🖵	**Brett E. Swierczewski** Silver Spring, MD, USA	Therapeutic Bacteriophage Cocktails for Compassionate Use against Multidrug-Resistant Infections	12+3 min

P1
Phage Therapy in Europe

Martha Clokie
University of Leicester, Department of Genetics and Genome Biology, Leicester, United Kingdom

Phage therapy is currently undergoing a revival of interest worldwide. It is particularly applicable to target diseases where bacteria are resistant to antibiotics or are in difficult to reach parts of the body that render antibiotic treatment ineffective. In the drive to reduce our reliance on antibiotic use, phage therapy can be contextualised within a newer concept of being a tool to modify the microbiome and selectively remove key pathogens.

Within Europe some countries, such as Georgia and Russia have a particularly long relationship with Phage Therapy, this is where the practice of using phages started and developed. France and Poland also have a long tradition of using Phage therapy which has continued to this day. More recently, Belgium has been advancing the practice both in terms of treating patients and by addressing regulatory issues. There have also been pockets of compassionate use in Germany and Switzerland. Other European countries such as Portugal and the UK have an active research scene, but this is not reflected in phages being used in patients.

In this presentation I will discuss how and where phages are being used for human therapy and highlight what disease indications they are generally used for. I will also discuss how and where phage collections and genomic data are stored and collated. I will review the limitations and breakthroughs that related to developing phage therapeutics and show how these are being addressed within Europe. Finally, I will contextualise human phage therapy work using phage therapy to target animals can inform human phage therapy development.

P2
Initial host range analysis of commercial phage preparations on Ukrainian MRSA isolates

Alla Yushchenko[1], Zinaida Klestova[1], and Tetiana Garkavenko[2]
1- State Scientific-Control Institute of Biotechnology

and Strains of Microorganisms, Kyiv, Ukraine; 2- State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Kyiv, Ukraine

Methicillin-resistant *S. aureus* (MRSA) is a major threat to public and animal health due to a lack of effective treatment options. Current elevated rates of MRSA infection warrant new therapeutic alternatives. In Ukraine, consistent with other geographical regions, increasing reports of MRSA infection have been reported. Moreover, MRSA strains have been illegally transported across state boundaries with subsequent interception by state agencies protecting public health. Use of bacteriophages represent a promising therapeutic, detection and prevention strategy with proven effectiveness against MRSA. However, more robust phage-based treatments with broad host range are needed. In addition, the development of novel phage-derived detection strategies is warranted. In this study, we evaluated the activity of two commercial phage preparations against a local collection of diverse community and food-associated MRSA host strains and characterized individual phages with enhanced efficacy. Our data revealed that the Ukrainian MRSA host strains showed resistance to both commercial preparations tested. Phage isolated from commercial preparation 1 (1SinB phage) was found to be ineffective against MRSA, while a single phage isolated from commercial preparation 2 (phage Nibbler 21) showed strong lytic activity via spot test against 6/10 local MRSA strains. We report here the first host range assessment of commercial phages on community and food-associated MRSA isolates in Ukraine. Further host range analysis on an expanded collection of Ukrainian MRSA isolates as well as taxonomic and genomic characterization of the Nibbler 21 phage is planned.

P3

Learning from bacteriophages how to overcome the resilience of antibiotic-tolerant, dormant bacteria

Enea Maffei, Yannik Heyer, Marco Burkolter, Aisylu Shaidullina, and Alexander Harms
University of Basel, Biozentrum, Basel, Switzerland

The current crisis of antimicrobial therapy is fueling a renaissance of "phage therapy" in which bacteriophages, the viruses that prey on bacteria, are used as therapeutics against multidrug-resistant pathogens. However, so far interactions of phages with their bacterial hosts have been studied primarily in laboratory experiments with a few model phages, while the reality inside patients or in natural environments remains poorly understood.

We therefore study how bacteriophages infect slow- or non-growing bacteria that dominate in most ecosystems and are a major cause of chronic or relapsing infections due to their notorious antibiotic tolerance. Our results and previous work show that the vast majority of bacteriophages including all tested standard laboratory models are either unable to adsorb to dormant hosts or merely enter a state of hibernation ("pseudolysogeny"). We therefore sampled diverse natural environments and screened for bacteriophages with the ability to directly replicate on dormant host cells. These experiments resulted in the isolation of *Paride*, a novel phage that uniquely wipes out deep-dormant cultures of *Pseudomonas aeruginosa* laboratory strains and clinical isolates irrespective of their extreme antibiotic tolerance. We are currently studying the molecular mechanisms underlying the ability of *Paride* to infect and kill dormant hosts as well as the inability of other phages to do the same. Our results will shed new light on the biology of bacteriophages in natural environments and contribute to a rational application of phage therapy. Furthermore, a molecular understanding of weak spots in the physiology of dormant bacteria exploited by phage *Paride* might also inspire new pharmacological treatment options for chronic bacterial infections.

P4

Isolation, characterization and engineering of lytic bacteriophage TUN1 specific for *Klebsiella pneumoniae* K64 clinical isolates from Tunisia

Jana Stender[1], Simone Eckstein[1], Sonia Mzoughi[2], Kilian Vogele[3], Jana Kühn[1], Daniela Friese[1], Christina Bugert[1], Susan Handrick[1], Mustafa Ferjani[4], Roman Wölfel[1], Andrew Millard[5], Mohamed Ben Moussa[6], and Joachim J. Bugert[1]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- Faculty of Pharmacy, Monastir, Tunis, Tunisia; 3- Technical University of Munich, Department of Physics, Garching, Germany; 4- Military Hospital of Instruction of Tunis, Department of Anesthesiology and Reanimation, Tunis, Tunisia; 5- Department of Genetics and Genome Biology, Leicester, United Kingdom; 6- Military Hospital of Instruction of Tunis, Department of Virology, Tunis, Tunisia

Multidrug-resistant *Klebsiella pneumoniae* spp. (*Kp*) are emerging agents of severe infections of the respiratory, urinary tract and wounds that can progress to fatal septicemia. The use of bacteriophages is currently being considered as an effective alternative or adjuvant to antibiotic therapy.

In this study, we report capsule (K)-typing of 163 carbapenem-resistant *Kp*(CRKP) isolated 2014-2018 at the Military Hospital of Instruction of Tunis (MHT), Tunisia, by partial amplification and sequencing of the *Kp wzi*gene. The most prevalent K-type overall was K64 with 50.3 % followed by K17 and K27 (22.7 and 11.0 %, respectively). K64 *Kp*strains were most common and associated with increased case/fatality rates, especially at the intensive care unit (ICU). Using a K64 *Kp* strain we isolated and characterized a lytic *Kp* phage, vB_KpP_TUN1 (phage TUN1), from wastewater samples of the ICU at the MHT. TUN1 belongs to the *Autographiviridae* family and specifically digests K64 *Kp*capsules via a depolymerase encoded by *gp47*. Furthermore, we successfully assembled phage TUN1 in a non-replicative host (*E. coli*) raising the possibility of in vitro assembly in the absence of live bacterial hosts. We propose that phage TUN1 is a promising candidate to be used as an adjuvant or an alternative to antibiotic therapy in CRKP infections, facilitating regulatory approval of phage therapy.

In summary, K64, K17 and K27 are the most common *wzi*capsule types in this geographical location in Northern Africa. The lytic phage TUN1 efficiently lyses K64 *Kp*strains associated with increased case/fatality rates at body temperature. Together with its ability to be rescued in a non-replicative host these features enhance the utility of this phage as an antibacterial agent.

We are currently investigating the factors of TUN1 that determine its host-specificity i.e. capsule depolymerases and receptor-binding proteins. Furthermore, we are working on genetically modified phages to generate i) reporter phages for rapid screening of host susceptibility and ii) highly effective recombinant phages for therapeutic purposes.

P5

Bacteriophages - Old antiinfectives with new perspectives

Holger Ziehr[1], M. Brack[2], C. Dasenbrock[3], D. Garbe[4], M. Häfner[5], S. Hebecker[1], R. Hertrampf[6], A. Hüser[6], I. Korf[1], B. Lang[7], A. Lakowitz[1], M. Müller[7], C. Rohde[8], A. Ross[1], Q. Schefer[6], C. Seitz[1], M. Stichling[5], W. Wenzel[7], S. Wienecke[1], S. Wienhold[2], C. Willy[5], J. Wittmann[8], and M. Witzenrath[2]

1- Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Braunschweig, Germany; 2- Charité-Universitätsmedizin Berlin (Charité), Department of Infectious Diseases and Pulmonary Medicine, and Division of Pulmonary Inflammation, Berlin, Germany; 3- Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany; 4- Department XXIV Hospital Pharmacy, Bundeswehr Hospital , Berlin, Germany; 5- Department Trauma & Orthopedic Surgery, Septic & Reconstructive Surgery, Research and Treatment Center for Complex Combat Injuries, Bundeswehr Hospital , Berlin, Germany; 6- Charité Research Organisation (CRO), Berlin, Germany; 7- Department XXI Clinical Microbiology & Hospital Hygiene, Bundeswehr Hospital, Berlin, Germany; 8- Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Bacterial pathogens are increasingly developing resistances to all groups of antibiotics. As consequence there are multiple cases of bacterial infection in which available antibiotics are no longer effective. This is the motivation to investigate supplements to classical antibiotic therapy. One promising method is the therapeutic use of bacteriophages.

Two therapeutic phage projects, *Phage4Cure* and *PhagoFlow* are actually performed by Fraunhofer ITEM in Braunschweig and its project partners.

Pseudomonas aeruginosa is one of the most frequently bacteria found in the lung of cystic fibrosis patients and leads to more progressive and severe course of disease. Since 2017 the project *Phage4Cure* is funded by Federal Ministry of Education and Research (BMBF). In this project three *P. aeruginosa* phages were isolated by German Collection of Microorganisms and Cell Cultures (DSMZ), propagated in host cells and purified for application in an inhalable cocktail. The phage API manufacturing is performed in compliance to pharmaceutical quality requirements i.e. Good Manufacturing Practice (GMP). Each phage drug product component is aseptically manufactured at Fraunhofer ITEM. The *Phage4Cure* phage cocktail will be used in a clinical trials (Phase I, IIa) in 2022. Currently, the manufacturing license extension is filed and waiting for approval.

The DSMZ, Fraunhofer ITEM Braunschweig and the Bundeswehr Hospital Berlin have started the joint project *PhagoFlow* in 2019, which is publicly funded by the Innovationsfond des Deutschen Bundestages. The aim of this project is to investigate manufacturability and quality requirements for magistral phage preparation in Germany for the treatment of patients with wound infections of the extremities. Magistral phage preparation represent customized formulation of phage cocktails individually tailored for patients infection. Target bacteria are pathogens of priority class 1 and 2 of the WHO list, for which new antibiotics are urgently needed. This project is performed in close alignment with the Federal Institute for Drugs and Medical Devices

(BfArM). Currently, manufacturing license is filed and the whole platform-like process sequence for *PhagoFlow* will be operational from quarter 3 2021.

P6

A cell-free platform for production, modification and engineering of bacteriophages for therapeutic use

Kilian Vogele[1], Peter Braun[2], Jana Stender[2], Sophie von Schönberg[1], Franziska Winzig[1], Quirin Emslander[3], Markus Joppich[4], Miriam Abele[5], Chen Meng[5], Christina Ludwig[5], Joachim J. Bugert[2], Christian Willy[6], Gill G. Westermeyer[7], and Friedrich C. Simmel[1]
1- TU München, Chair of Physics of Synthetic Biological Systems (E14), Garching, Germany; 2- Bundeswehr Institute of Microbiology, Munich, Germany; 3- Technical University of Munich, Institute of Virology, Munich, Germany; 4- Ludwigs-Maximilian-Universität München, Department of Informatics, Munich, Germany; 5- Technical University of Munich, Bavarian Center for Biomolecular Mass Spectrometry, Munich, Germany; 6- Bundeswehr (Military) Academic Hospital Berlin, Berlin, Germany; 7- Helmholtz Zentrum München, Institute of Biological and Medical Imaging, Oberschleißheim, Germany

Bacteriophages are a promising therapeutic approach to combat rapidly increasing numbers of infections with multidrug-resistant bacteria. However, broad implementation of bacteriophage therapy is currently impeded by a lack of safe production standards and insufficient phage characterization.

We utilized a cell-free expression system to produce high titers of bacteriophages in a one-pot reaction, fully omitting the use of living bacteria. We developed this system to a host-independent platform for the production of phages against both gram-positive and gram-negative bacteria.

At a microliter-scale, our *E. coli* derived cell-free expression system produces effective doses of phages against *E. coli,* including enteroaggregative *E. coli* (EAEC), *K. pneumonia, Pseudomonas sp.* and *Y. pestis.* By co-expressing suitable host factors, we were furthermore able to extend the range of our platform to include phages of gram-positive bacteria like *B. subtilis.* We also introduce a method for transient, non-genomic phage engineering, that allows the addition of functionalities for a single replication cycle. For that we suppressed the expression of a phage capsid protein in the cell-free reaction while at the same time expressing a modified version from a different DNA template, to generate transiently modified phages.

Finally, we showcase a pipeline for personalized phage therapy of a multidrug-resistant ESKAPE pathogen from isolation to *in vitro* production of phage against a clinical isolate of *K. pneumonia.* We expect our cell-free methodology to enable accelerated reverse and forward phage engineering as well as the safe and customized production of clinical-grade therapeutic bacteriophages.

P7

Therapeutic Bacteriophage Cocktails for Compassionate Use against Multidrug-Resistant Infections

Andrey Filippov, Helen Freyberger, Yunxiu He, Amanda Ward, Nino Mzhavia, Yoon Kwak, Min Tong, Patrick Mc gann, Jason Bennett, Stuart Tyner, Damon Ellison, Derese Getnet, Mikeljon Nikolich, and Brett E. Swierczewski
Walter Reed Army Institute of Research, Bacterial Diseases Branch, Silver Spring, MD, USA

Background: The ESKAPEE pathogens cause various multidrug-resistant (MDR) infections that are challenging to treat. Bacteriophages (phages) are one of the most promising alternatives to antibiotics, as has been demonstrated by the results of several recent clinical trials and expanded access therapy in a number of clinical cases. The purpose of this work was to analyze the results of activity of our phage panels against clinical isolates of ESKAPEE bacteria, formulate custom cocktails, purify and test these cocktails, and enable FDA clearance for therapeutic application by attending physicians.

Methods: Phage susceptibility testing was performed using an efficiency of plating assay and only phages with sequenced genomes were selected as potential therapeutics. Phages were purified from endotoxin by overnight high-speed centrifugation in cesium chloride gradients, chromatography using Detoxi-Gel Endotoxin Removing Gel (Thermo Scientific, USA) and EndoTrap (Hyglos, Germany) columns, dialysis against 1,000 volumes of PBS-Mg (phosphate-buffered saline with 10 mM of magnesium sulfate) and filter-sterilization. Phages for custom cocktails were aseptically mixed and filter sterilized. Phage single doses (10 extsuperscript9 PFU per 1 mL of the cocktail) were vialed in sterile non-pyrogenic cryogenic tubes. Sterility testing was performed with the rest of the bulk phage mix in a liquid medium and on agar plates for 72 hours in-house and per USP <71> for 14 days by contract with a private company. Phage potency, purity (including endotoxin and exotoxin A content) and sterility results were reported to the FDA by the attending physician.

Results: A 5-phage cocktail, PPM1, was used against a severe *P. aeruginosa* knee wound infection. After intravenous (IV) treatment during six weeks, no *P. aeruginosa* infection was detected in the patient. A cocktail of four phages, PPM3, was administered intravenously to an elderly patient with a *P. aeruginosa* ventricular assist device infection, which initially caused a striking improvement of the patient's condition and clearance of the bacteria. The third cocktail that consisted of three phages, PPM2, was used against a *P. aeruginosa* aortic graft infection that resulted in complete clearance of the infection. Two unique *E. coli* phages isolated in our lab are in current use for the treatment of recurrent bacteremia with unknown source of infection. More randomized clinical trials are needed to for bacteriophage therapy.

Audimax / 12:30 ... 13:00

Q

Poster awards and farewell

Chairs: Roman Wölfel (DEU), Arne C. Rodloff (DEU), and Herbert P. Schweizer (USA)

BP1

Evaluation of Biological Warfare Agent Clinical Sampling and Analysis

Robert L. Cubeta
Institute for Defense Analyses, Alexandria, VA, USA

In the absence of effective environmental sampling, the military medical system will likely provide the first indication of a biological warfare attack. Accurate and timely diagnostic results provide critical situational awareness that informs the implementation of disease response measures, revised planning, and treatment of ill individuals. The Institute for Defense Analyses evaluated how various tactics techniques and procedures for the collection and analysis of clinical specimens influence the timing and accuracy of diagnostic test results. The analysis included a scientific literature review, the results of which we used to characterize the times during a given disease's progression that a given diagnostic test can generate accurate results. For multiple combinations of diseases and diagnostic technologies of interest, we were unable to find sufficient data to characterize when during the course of illness the diagnostic technology would generate accurate results. Of the disease and diagnostic technology combinations of interest for which sufficient data was available, high test sensitivity at or before symptom onset was not common. To further assess the disease and technology combinations that we found sufficient data on, we developed a stochastic individual based model to simulate disease progression, patient movement, and clinical specimen collection and analysis following a biological exposure event. We then analyzed the modeling results to determine how changes in tactics, techniques, and procedures for specimen collection and analysis affect the timing and accuracy of diagnostic results.

BP2

How can IoT improve prevention of Food-Borne Outbreaks - SIPA Study case

Samuel C. Migueis[1], Sérgia M. Pimenta[2], and Pedro T. Silva[2]
1- Academia Militar - CINAMIL, UMMV, Lisbon, Portugal; 2- Academia Militar, UMMV, Lisbon, Portugal

The last decades bring to Humankind new tools to prevent and improve health with the exponential growth of technology, mainly in the Internet of Things (IoT). Food Safety Management System (FSMS) based on HACCP seven principles improves consumers' safety, mainly after 2002-2004. Since that year, all Food business operators in European Community must put in place, implement and maintain permanent procedures based on HACCP principles.

Food operators at EC must fulfil the HACCP seventh principle: "establishing documents and records commensurate with nature and size of the food business to demonstrate the effective application of the measures outlined in (...)" principles 1 to 6. These goals are made mainly with paper records that drive the analysis and improvements complex and may target wrong and false registrations.

The IoT brings a unique opportunity to take new inputs into the data and handle it faster and more efficiently. These can improve the accuracy of risk assessment that can be improved and made just in time.

SIPA project (Sistema Integrado de Segurança Alimentar - Food safety integrated System) starts studying the impact of sensors in risk assessment in an industrial kitchen. With the implementation of some intelligent and autonomous sensors, the SIPA team wants to generate an algorithm that can predict the risk just in time during the production process.

BP3

Air Safety Planning: Structured risk analysis and management for natural or man-made air-associated health hazards

Manuel Döhla[1], Christin Döhla[2], and Martin Exner[2]
1- Bundeswehr Central Hospital Koblenz, Department of Microbiology and Hospital Hygiene, Koblenz, Germany; 2- University of Bonn, Institute for Hygiene and Public Health, Bonn, Germany

Background: The SARS-CoV-2 pandemic has highlighted the relevance of air hygiene in healthcare facilities and public buildings. While indoor air technology has so far played a transmission-preventing

role mainly in operating theatres, it is now apparent that building owners and operators of healthcare facilities, but also public buildings, will have to pay more attention to airborne obligate pathogens such as SARS-CoV-2 in the future.

Not only viruses, but also other natural air-associated health hazards (like pollen, fine dust, moulds, or heat) will become more important in the future; as possibly will man-made hazards (chemical or biological weapons).

Aim und Method: The purpose of this submission is to present a structured framework concept for the development and establishment of an air hygiene concept that follows the principles of "Hazard Analysis and Critical Control Points" (HACCP, food) and "Water Safety Planning" (WSP, drinking water). Therefore, we will refer to it in parallel as "Air Safety Planning" (ASP).

The cyclic ASP concept for buildings consists of six steps: *(1)* The establishment of an air hygiene team. *(2)* The description of the air handling system within the building. *(3)* The identification and analysis of hazards, hazardous events, risks, and existing control measures. *(4)* The development and implementation of a measure improvement plan. *(5)* Monitoring of the measures and control of the efficiency of the ASP. *(6)* Documentation, periodic review, and continuous improvement of all the above steps.

Conclusion & Outlook: Owners and operators of healthcare facilities and public buildings have not systematically considered air hygiene so far. Through ASP, existing as well as new buildings can in future be hardened against air-associated health hazards through technical, organisational and personal protective measures.

The health challenges of the present and future include climate change, new pathogens with epidemic or pandemic potential, and the risk of terrorist or criminal attacks.

ASP represents a structured risk management to counter these threats. It aims to achieve "healthy air" as result of structural and process quality improvements. It is all-encompassing, sets out options and priorities for action in a comprehensible way and leads to adaptation to new or changed hazards through periodic reviews.

BP4

Data stewardship - data management as a requirement for prevention and control of outbreak scenarios

Christin Döhla[1], Manuel Döhla[2], and Jens Dierkes[1]

1- University of Cologne, Research and Publication Support, Cologne, Germany; 2- Bundeswehr Central Hospital Koblenz, Department of Microbiology und Hospital Hygiene, Koblenz, Germany

Aim: Facing outbreak scenarios like the corona pandemic, it's all about data. These data include routine as well as research data from medical, microbiological, and epidemiological sources. In these complex outbreak scenarios, it would be helpful to have re-usable data from both, pre- and peri-outbreak periods. During a pandemic previous awareness, skills and good practices for Data Management (DM) may not be readily available. Therefore, it is challenging to generate and store FAIR data (Findable, Accessible, Interoperable and Re-usable). Data Stewardship (DS) serves as a concept for better DM through the whole data lifecycle. This cycle consists of six iterative parts: Planning Data, Analyse Data, Register Data, Publish Data, Preserve Data, Re-Use Data.

Background: DS is a process that makes one deal responsibly with one's own and other people over the whole cycle. DS as a complex intervention with a coordinating and supporting role to tackle this challenge. This goes smoothly by spanning boundaries between researchers, facilities, administrations; this is embedded in a German National Research Data Infrastructure for personal health data (NFDI4Health). DS can improve outbreak management and can optimate data as a source for guiding medical and political decisions. Therefore, these data can serve as a scientific quality control for measures that were implemented during a pandemic, but they can also serve as control data for upcoming pandemics in the future. Thus DS could help to train and to improve skills and methods for using data in a FAIR-way in the long-term.

Discussion: Personal health data are sensible data, their generation and storage are restricted by national and European regulations. In pandemic situations, there is a conflict between individual patient rights and community bio defensive interests. Outbreak DM with "health related Big Data" will give the chance for a systemic monitoring and for a more sensible alert threshold regarding natural outbreaks or man-made biological attacks. These positive effects are only achievable if the public, and in the end everyone, sees the chance of a better protection for all by entrusting their personal health data to DS. First results can be expected after first activities to order strengthen DS, inter alia training and cooperation of DS and biomedical researchers. Their feedback and needs in practice will be important for a more effective DS and re-use of data in a FAIR way.

BP5

[Contribution withdrawn]

BP6

Experience in the participation of the CBRN Defense Systems Department of the INTA-La Marañosa Campus (Spain) in projects of the European Commission ISF-P: BULLSEYE and MALL-CBRN

María V. Ortega, Juan M. Moreno, Olga Bassy, and Juan C. Cabria
1- INTA, CBRN, San Martín de la Vega, ESP

The CBRN Defense Systems Department of the INTA-La Marañosa Campus (Spain) currently participates in two European consortia for the calls of the Internal Security Fund-Police (ISF-P) instrument of the European Commission: the BULLSEYE and MALL-CBRN projects. The first of them aims to improve the preparation and response of European emergency services to chemical and biological incidents, while the second aims to create a protection system against CBRN and explosives incidents in large shopping malls. In both, the methodology is based on gaps analysis, and it is also supported by state of the art research, through interviews, meetings with experts, and the organization of workshops and training exercises. The main expected results in relation to the BULLSEYE is the provision of highly trained first responders who can then serve as trainers for their respective teams in their country of origin. In relation to the MALL-CBRN, one of the most important results will be the development of recommendations to improve prevention and response to these types of incidents, including those related to food.

CP1

Agent-based simulation as an effective tool for COVID-19 anti-epidemic policy formation

Dmytro Chumachenko[1], Ievgen Meniailov[1], Kseniia Bazilevych[1], and Tetyana Chumachenko[2]
1- National Aerospace University Kharkiv Aviation Institute, Mathematical Modelling and Artificial Intelligence, Kharkiv, Ukraine; 2- Kharkiv National Medical University, Epidemiology, Kharkiv, Ukraine

The COVID-19 pandemic has become a problem for health systems and predicting the dynamics of cases has become an indispensable tool for calculating the necessary capacity of medical institutions, the supply of personal protective equipment, medicines, ventilators, and the like. Mathematical modeling tools can be an effective solution to control the epidemic. With the help of modeling, it is possible to identify the factors that most affect the dynamics of the epidemic process in a certain area, and to regulate them. We hypothesize that agent-based approach will reveal the significance of factors affecting the epidemic process, such as quarantine restrictions, isolation of patients, the use of masks and antiseptics, adherence to vaccination, over current models that only allow to forecast the morbidity. We present our results of an agent-based model which is a set of interacting agents with different characteristics (age, sex, quantity of contacts per day, etc.) and different states (Susceptible, Infected, Exposed, Recovered, Dead). Transitions between states are implemented probabilistically and are determined experimentally based on statistics on the COVID-19 incidence. We used data on COVID-19 morbidity in Ukraine provided by Center for Public Health of Ministry of Health of Ukraine. We used C# programing language for program realization. The developed agent-based model of COVID-19 allows calculating the predicted incidence of COVID-19 in the regions of Ukraine based on real statistical data. The advantage of the approach is the ability to identify factors affecting the dynamics of the incidence of COVID-19, in contrast to classical and neural network models. While other models only allow constructing predictive incidence rates, the extended model makes it possible to conduct experiments and determine the leading driving forces of the epidemic process. This is the basis for developing optimal management decisions to help minimize the risk and increase the effectiveness of the epidemic response. The accuracy of predictions obtained using our model is 97.6%, which is higher than the compartment models widely used for modeling COVID-19. Experiments provided with agent-based model showed that the most effective measure for susceptible population to reduce the epidemic incidence of COVID-19 is contact tracking with isolation of patients and contact persons. At the same time, there is no need for complete isolation of the population.

CP2

Biological Threats and Special Pathogens - Why is a Quality Management beneficial?

Sophia Brünschwitz and Janine Kleymann-Hilmes
Robert Koch-Institut, ZV 6.2 Quality Management, Berlin, Germany

The Robert Koch Institute (RKI) is Germany's central governmental scientific institution in safeguarding public health and infection protection

in Germany. Together with partner countries it is part of international networks, exchanging information and advice to improve, amongst others, the preparation of disease outbreaks and health crises. A quality management system (QMS) is of significant importance to a federal institution, that performs specialized diagnostics. It becomes even more essential with task areas, being involved with the defense of biological hazards and outbreaks of highly pathogenic or bio terroristic agents. During the latest international pandemic, the awareness of the significance of a QM in medical laboratories did a decisive leap. Currently quality assurance and moreover a QM are a matter of public and political interest. The population, the public health service and politicians expect quality-assured methods and procedures as a basis for recommendations from a Public Health Institute. The RKI is accredited to deal with biological threats and special pathogens along with its various other diagnostic laboratories and service facilities according to three different norms: ISO 15189 (medical laboratories), ISO/IEC 17025 (testing laboratories) and ISO/IEC 17043 (proficiency testing). In this connection ISO/IEC 17043 is especially used to deal with highly pathogenic living Risk Group 3 bacteria. Moreover, the RKI's specialized laboratory for highly pathogenic viruses is accredited for SARS-CoV-2 with a flexible scope. Due to the flexible scope other highly pathogenic viruses, e.g. Marburg virus, Zika virus and Ebola virus, are currently verified whether they can be included in the scope of the accreditation.

Besides the RKI other institutes own a professional QMS with accreditations for highly pathogenic organisms as well. The Friedrich-Loeffler-Institute with a focus on farm animal health and welfare and on the protection of humans from zoonoses is accredited according to ISO/IEC 17025 and the Bundeswehr Institute of Microbiology is accredited according to ISO 15189. Through an accreditation they proof their reliability of their diagnostics not only in Germany but globally.

This paper is supposed to give an overview of the QM of biological threats and special pathogens at the RKI.

CP3

Epidemiological surveillance of COVID-19 during March-May 2020 in Armenia

Karine M. Gevorgyan[1], Lilit M. Avetisyan[2], Lusine V. Paronyan[1], Gayane G. Melik-Andreasyan[3], and Artavazd V. Vanyan[4]
1- NCDC, Zoonotic and Parasitic Epidemiology department, Yerevan, Armenia; 2- NCDC, Deputy Director-General, Yerevan, Armenia; 3- NCDC, Department of Scientific programs NCDC , Yerevan, Armenia; 4- NCDC, Director-General, Yerevan, Armenia

Introduction: In the first weeks of the pandemic, in Armenia, only patients with an epidemiological link and clinical signs were examined. The aim of this work was to assess the sensitivity of the surveillance system and risk factors of fatality in age/sex groups during March-May.

Methods: We reviewed positive PCR cases (4,532) diagnosed in March-May 2020. Epidemiological data were analyzed using the ARMED information system.

Results: The sensitivity of the surveillance was low because the system did not include patients with severe complications if their nasopharyngeal PCR was negative. PCR testing of feces or bronchial fluid were not performed. Only positive PCR cases were hospitalized and treated as COVID-19. Negative PCR cases with mild infection were isolated at hotels with more severe patients isolated in their homes and treatment provided by polyclinics. Negative PCR cases are not included in the ARMED information system.

The distribution of fatality rates by age and sex are described. During March-May 2020, there were 4,532 total cases with a 4.5% fatality rate. In the $\geq$ 80 age group, total cases 232, fatality rate 25.0% (male 36.7%, female 16.4%); In the $\geq$ 70 age group, total cases 441, fatality rate 14.1% (male 21%, female 9.5%); 60-69 age group cases 1285, fatality rate 3.9% (male 6.5%, female 2.3%); 50-59 age group, cases 1321, fatality rate 2.1% (male 3.4%, female 1,5%); 40-49 age group, cases 1253, fatality rate 0.4% (male 0.5%, female 0.4%). The fatality rate is correlated with age/sex and older age groups. In the same age groups, the male gender was identified as a risk factor. Additionally, there was a decrease in fatality rate from March to April (4.2% to 3.1%) and an increase from April to May (3.1% to 5.1%).

Conclusions: The sensitivity of the surveillance system was low and can be enhanced by increasing the total number of PCR tests by expanding the type of tested samples (feces, bronchial fluid). Also, it is recommended to include ELISA and rapid tests to increase the sensitivity of surveillance to avoid underreporting of cases. It is necessary to strengthen control for Covid-19 cases, considering the older age and male gender as risk factors. Further research is necessary to identify the reasons for the increase in fatality rate.

CP4

Impact of COVID-19 pandemic on mental health: Online survey in Armenia

Ani Manukyan[1], Lilit Avetisyan[2], Adela Paez[3], Gayane Sahakyan[2], Lusine Paronyan[1], and Artavazd Vanyan[4]
1- NCDC, Zoonotic and Parasitic Epidemiology department, Yerevan, Armenia; 2- NCDC, Deputy Director-General, Yerevan, Armenia; 3- Medipiet Scientific Coordinator, Madrid, Spain; 4- NCDC, Director-General, Yerevan, Armenia

Introduction: The COVID-19 pandemic is a global health challenge with cases of COVID-19 registered worldwide. According to official data, in Armenia, 7.1% of the population (as of April 20, 2021) have been infected. Infectious disease outbreaks, like COVID-19, can be scary, stressful and can affect mental health. Quarantine, long-term isolation, and restrictions can lead to disorders of human psychology. The pandemic has affected the lives of general population and they are at risk of mental illness. This study aimed to assess and better understand the potential impact of the COVID-19 pandemic on mental health.

Materials and methods: A cross-sectional web-based survey was carried out from January 5 to March 15, 2021, among the adult population. Respondents were recruited using a Facebook platform linked to an online google form. People were asked to answer a self-administered questionnaire and a SRQ-20, which is a 20-item self-report screening tool. EpiInfo v.7 was used for data entry and cleaning and STATA 16 Software for data analysis.

Results: Response rate=100% (n=384). The main respondents were from Yerevan city (63%), Kotayq Region (7.3%), Ararat Region (5.2%). The majority of respondents were women (81.2%, n=312). Covid-19 has been diagnosed among 36.5% of participants. Participants have no mental health condition previously (97.1%) and 69.8% have no health issues. Participants also reported feelings after seeing the news: 44.5% were unaffected/not concerned, 40.6% depressed/anxious, 11.3% helpless, 3.6% it's the end of the world. Cronbach's alpha (reliability of SRQ-20 items) = 0.81, which suggests a good internal consistency that items are related to each other. Of the respondents, 121 (31.5%) screened positive for mental health problems as per SRQ-20 score, of which 109 (28.4%) were females and presented higher scoring of SRQ-20 between 18-39 years of age. The mean of SRQ-20 total score is 5.27.

Conclusions: Effects of COVID-19 on mental health should be a public health priority. Credible sources of information are key to avoid the fear and panic that misinformation may cause and uncertainty surrounding an outbreak. Females are at higher risk of developing mental health problems. In Armenia, men generally do not share emotional problems and internalize them. Creating a mental health support program during a time of public health emergencies is advised.

CP5

Evaluation of infection prevention and control programs in Armenian hospitals in the era of COVID-19 pandemic - Armenia, 2021

Gennady Palozyan[1], Shoghik Avetisyan[1], Romella Abovyan[1], Gayane Melik-andreasyan[2], and Artavazd Vanyan[3]
1- NCDC, MoH of Armenia, Department of Epidemiology of Infectious and Non-Communicable Diseases, Yerevan, Armenia; 2- NCDC, MoH of Armenia, Reference Laboratory Center, Yerevan, Armenia; 3- NCDC, MoH of Armenia, Yerevan, Armenia

Introduction: Infection prevention and control (IPC) is important for both patients and medical staff safety, especially in the era of COVID-19 pandemic. The WHO released the Infection Prevention and Control Assessment Framework (IPCAF) tool for assessment of IPC structures at the facility level through eight core components. The aim of this study was to evaluate implementation of IPC programs in the hospitals of Armenia.

Methods: The National Center for Disease Control and Prevention of the Ministry of Health (MoH) of Armenia sent a translated version of the IPCAF questionnaire to all 119 hospitals of Armenia. Data was collected during 10/2020-02/2021. We calculated score results for each hospital as well as the averages for each country regions and for the whole country.

Results: The average score for all hospitals was 544.3, which corresponds to "intermediate" IPC level. Out of 119 Armenian hospitals, 32 hospitals correspond to "advanced", 79 to "intermediate", 8 to "basic" with no hospitals considered at the "inadequate" level of IPC. The average score for hospitals in the capital was higher, than for regional hospitals (average score 585.5 vs 514.4). High scores were received only for core component 2 (CC2) (IPC guidelines) and CC8 (Built environment, materials, and equipment for IPC at facility level). The lowest score was received for CC5 (Multimodal strategies).

Conclusion: This study showed the necessity to improve a majority of key core components, especially in the regional hospitals. Multimodal strategies and Health-care associated infection (HAI) surveillance should be prioritized for national implementation.

Education and structured continuous training programs should be developed to support implementation at all levels.

CP6

Implementing an in-house-developed automated data analysis pipeline supported by a commercially available software solution to generate NGS reports with epidemiological analysis combined with prediction of clinically relevant antibiotic resistance determinants

Mike H. Pillukat, Maximilian Klepsch, Alexandra Rehn, Malena Bestehorn-Willmann, Josua Zinner, Peter Molkenthin, Mathias Walter, and Markus Antwerpen
Bundeswehr Institute of Microbiology, FMG, Munich, Germany

Sequencing and timely processing of NGS data from clinically relevant multi-drug resistant species can be performed very efficiently using today's advanced hardware and software solutions. However, presentation of the final results in a format that is easily understandable and relevant for clinicians as well as for infection control personnel and antibiotic stewardship team members is often lacking in most commercially or in-house bioinformatics solutions.

In this proof-of-principle study, we aimed at bridging this gap using a sample data set of sequence data from glycopeptide-resistant enterococci isolated from clinical material.

NGS sequencing, assembly and annotation of bacterial DNA sequences was performed as described previously (see Antwerpen et al., Microorganisms 2019, 7 (12), 689). For management of sample sequence data, calculation of the distance matrix and antimicrobial resistance factor detection, the commercially available software solution SeqSphere® (Ridom GmbH; Muenster, Germany) was used. An in-house automated downstream analysis pipeline was scripted in R Studio® (RStudio, PBC; Boston, Massachusetts, U.S.A.). Report generation was performed by R Studio® scripts using guidelines established by an infection control and antibiotic stewardship specialist.

Automated report generation including epidemiological analysis and antibiotic resistance forecast from NGS data from a clinically relevant multi-drug resistant gram-positive bacterial species provides an easily adaptable template for analyzing clinical samples from other gram-positive as well as gram-negative species.

Acknowledgments: For provision of extracted genomic DNA from a representative set of vancomycin-resistant *Enterococcus faecium* clinical isolates the authors thank Dr C. Balczun and Prof Dr P. Scheid of the Central Laboratory of the Bundeswehr Central Hospital in Koblenz, Germany.

CP7

Characterization of culture-independent screening methods of the detection of *L. pneumophila* in artificial water systems

Philipp Streich[1], Björn Biedermann[2], Johannes Redwitz[3], Lück Christian[4], and Michael Seidel[1]
1- TUM, Chemistry, Munich, Germany; 2- rqmicro, Schlieren, Switzerland; 3- LGL, Munich, Germany; 4- TUD, Medical Microbiology and Hygiene, Dresden, Germany

Legionella are gram-negative bacteria, which contaminate under certain circumstances cooling towers, evaporative coolers and wet separators. For humans, the inhalation of *Legionella* containing bioaerosols can cause bronchial pneumonia called legionnaires' disease with a mortality rate up to 10%. In several cases, those artificial water systems can release such contaminated aerosols over several kilometres. In Germany, the 42. BImSchV regulates the microbiological exposure of artificial water using the culture method, which takes up to 10 days for analysis. To establish rapid quantification for artificial water systems the validation of culture-independent methods is necessary. The aim of the project LegioRapid is to establish environmental experts laboratories who can quantify rapidly *Legionella spp* and *L. pneumophila.* Rapid methods are classified in molecular biological like integrity-qPCR, and antibody-based assays like immunomagnetic separation coupled with flow cytometry or the chemiluminescence sandwich microarray immunoassay. All methods need to be combined with enrichment methods to achieve quantitative data in range of defined action levels.

A standardized protocol was defined, to qualify rapid methods as quantitative or qualitative screening methods. The validity of the protocol was tested in 3 laboratories. In the first phase, the water matrix will be spiked with a known *Legionella* concentration to verify the efficiency, reproducibility, and robustness of every method. Calibrations were performed in EVIAN water as standardized matrix. Afterwards, a more complex water chemistry was used which simulates water of artificial water systems. In the next phase of the study, biocides were additionally added to the EVIAN water with water chemistry to generate an as realistic as possible matrix. In the final phase, every method will be used for the analysis of up to 200 real samples. Further-

more, during the whole validation study, the culture method will be done concurrently to investigate the correlation to each method.

In the future, the culture-independent methods should be used for more effective risk assessment and outbreak management for *Legionella* contamination in cooling towers, evaporative coolers and wet separators. The validation protocol will be published in the VDI guideline 4520 - part 2 and can be used for establishing an expert laboratory that uses cultivation independent methods.

DP1

Seroprevalence of anti-Hantavirus- und anti-Leptospira-IgG among Forestry Workers in Lower Saxony, Germany: Risk factors and risk communication towards at-risk groups

Christiane M. Klier, Christina Princk, Maren Mylius, Masyar Monazahian, Sophie Rettenbacher-Riefler, Armin Baillot, and Johannes Dreesman
Public Health Agency of Lower Saxony, Hanover, Germany

Hantavirus-infections and Leptospirosis are re-emerging zoonotic diseases. Pathogen-specific seroprevalences and risk factors related to leisure and occupational exposures among an at-risk group of forestry workers (FW) in Lower Saxony (LS) were studied. Findings will be used to develop both targeted public health measures and communication strategies for dissemination.

Sera sampled in 2016 among FW in LS were screened for anti-Hantavirus IgG (H) and anti-Leptospira IgG (L) by ELISA. Data on potential risk factors were collected by standardized questionnaire and analysed by multivariable logistic regression. Odds ratios adjusted for age and sex (aOR) and 95% confidence intervals (95%-CI) are reported.

Out of 601 FW (89.4% male, median age 53ys) 8.8 % were H-positive; 4.9 % L-positive. Seropositivity was higher in male than female FW (p= 0.04). One H-positive FW (n=53) recalled clinical diagnosis; none of the L-positive FW (n=27) did.

Taking walks (aOR: 0.3; 95%-CI: 0.1 - 0.9) and keeping pet cats (0.5; 0.3 - 0.9) decreased the chance of H-positivity; working outdoors (2.8; 1.3 - 6.4), keeping reptiles (3.1; 1.2 - 8.5), cleaning attics (2.4; 1.1 - 5.4) and canoeing (2.4; 1.2 - 4.6) increased the chance of H-positivity. Swimming in freshwater (2.7; 1.4 - 5.2) increased the chance; higher educational degree (0.6; 0.5 - 0.9) and gardening > 10ys decreased the chance of L-positivity (0.3; 0.1 - 0.99). FW obtained pathogen-specific information primarily from employers and colleagues. Seroprevalences are within the range of other studies among FW in Europe. Findings indicate that awareness needs to be raised among physicians, at-risk groups and the public towards these diseases Targeted prevention measures related to leisure and occupational risk e.g. "use masks when cleaning attics" needs to be developed. Both employers and public health institutions are key players in disseminating information and preventive measures. Development of targeted communication strategies to reach out to physicians, at risk-groups and the public is of importance.

DP2

ZOOSEQ- Establishment of Direct RNA Sequencing at the Bundeswehr Institute of Microbiology

Malena S. Bestehorn-Willmann, Max Klepsch, Alexandra Rehn, Matthias C. Walter, and Markus Antwerpen
1- Bundeswehr Institute of Microbiology, Munich, Germany

Classic sequencing methods are based on technologies that require DNA as input material. Therefore, double stranded cDNA has to be generated first out of RNA samples, which already induces alterations to the original nucleic acid sequence information. Depending on the method of first strand synthesis, information about RNA integrity, subgenomic RNA structures and biochemical modifications evade detection. Many methods of library preparation further require subsequent PCR steps that may additionally modify the sequence by introducing INDELs or mutations. These issues can be addressed by direct RNA sequencing using the nanopore sequencing method provided by Oxford Nanopore Technologies. The nanopore structure can be used for either DNA or RNA sequencing and only requires the attachment of a protein-bound adapter that initiates sequencing of a single nucleic acid strand. Because no further alteration of the RNA/DNA molecules is performed, information about sequence integrity and biochemical modifications can be resolved.

During this project two different methods of direct RNA sequencing were investigated: First, a target specific approach applying a custom designed adapter was used to enrich for whole genome RNA virus sequences. And second, a ubiquitous RNA sequencing approach was used without prior to cDNA synthesis. Therefore, only a poly A-tail was attached to all RNAs of the sample and afterwards sequenced using the poly-T Adapter provided by ONT. With this method information about integrity, subgenomic RNAs and cellular RNAs can be de-

tected without any bias based on amplification steps.

As target agents we used two plus strand RNA viruses, tick-borne encephalitis virus (TBEV) and SARS-CoV-2 as well as one negative strand RNA virus, Puumala virus.

We were able to acquire whole genome sequences with increased coverage by sequencing the complete RNA of virus cultures in combination with de-novo-assembly using reads of all sizes. In addition, using the target specific approach, we were able to acquire limited numbers of whole genome sequence reads for TBEV and SARS-CoV-2 in one read.

As the quantity of whole genome RNA sequences even from virus cultivation samples is limited, RNA purification and library preparation methods have to be further improved to address this issue in order to generate whole genome sequences reads in satisfying numbers, even from clinical or complex matrices.

DP3

Tick species and their pathogens from cattle in the Adama Region of Ethiopia

Lidia Chitimia-Dobler[1], Tafese beyene Tufa[2], Gerhard Dobler[1], Silke Wölfel[1], and Deon Bakkes[3]
1- Bundeswehr Institute of Microbiology, Virology and Rickettsiology, Munich, Germany; 2- Arsi University, Collegde of Health Science, Asella, Ethiopia; 3- Stellenbosch University, Dept. of Botany and zoology, Stellenbosch, South Africa

Introduction: Eastern Africa is a politically instable area in our world. Therefore, it is a target for stabilizing and peace missions of various armed forces of the European Union and NATO. Eastern Africa is an area of distribution of different vectors and their pathogens which might pose a health risk to soldiers and also to service dogs. So far, only limited data are available on the tick species prevalent in this areas and the pathogens they carry and potentially may transmit to humans and animals.

Methods: In 2016, 291 ticks were collected from 37 bulls in Adama, Ethiopia, which were ready for slaughter. Ticks were identified morphologically. Total genomic nucleic acid was extracted from ticks and used to test for molecular species identification and for testing against *Rickettsia* spp. and Crimean-Congo Hemorrhagic Fever virus (CCHFV) by real-time PCR. Species identification was done by phylogenetic analysis using sequencing that targeted the 23S-5S intergenic spacer region and *ompA* genes.

Results: Four tick species from two genera, *Amblyomma* and *Rhipicephalus*, were identified. *Amblyomma cohaerens* was the dominant species (n=241, 82.8%), followed by *Amblyomma variegatum* (n=22, 7.5%), *Rhipicephalus pulchellus* (n=19, 6.5%), and *Rhipicephalus decoloratus* (n=9, 3.0%). Among all ticks, 32 (11%) were positive for *Rickettsia* spp. and 15 (5.2%) of these were identified as *R. africae* comprising at least two genetic clades, occurring in *A. variegatum* (n=10) and *A. cohaerens* (n=5). The remainder of *Rickettsia*-positive samples could not be amplified due to low DNA yield. None oft he ticks were found positive for CCHFV.

Conclusions: The present study identified tick species which attack humans and animals and transmit pathogens of medically and veterinarily importance. No CCHFV was detected which speaks against a role of these most prevalent tick species in the region as vectors for this virus. However, the high prevalence rate of *Amblyomma* ticks for *R. africa*, the etiological agent of African tick bite fever, implies a high risk of infection for soldiers in the respective region. These data show the relevance for a One-health approach for monitoring and prevention of tick-borne disease risk and transmission.

DP4

Protection of tick-borne encephalitis (TBE) vaccines against the alimentary route of TBE transmission

Gerhard Dobler[1], Thomas Hierl[2], Markus Antwerpen[3], Marco Drehmann[4], Alexander Lindau[4], Rainer Oehme[5], Ute Mackenstedt[4], and Lidia Chitimia-Dobler[1]
1- Bundeswehr Institute of Microbiology, Virology and Rickettsiology, Munich, Germany; 2- Public Health Office, Tübingen, Germany; 3- Bundeswehr Institute of Microbiology, Munich, Germany; 4- University of Hohenheim, Dept of Parasitology, Hohenheim-Stuttgart, Germany; 5- State Public Health Office of Baden-Württemberg, Stuttgart, Germany

Introduction: Tick-borne encephalitis (TBE) is the most important tick-borne virus infection in Europe and Asia. Besides the transmission by ticks there is another route of transmission of TBE virus the alimentary route by raw milk and milk products, which has been mainly observed in Eastern Europe, but during the last years was sporadically found also in Germany. So far it has never been proven that the available TBE vaccines are able to protect from alimentary transmitted TBE virus infection.

Methods: In May 2017, an TBE outbreak linked to alimentary infection by raw milk from a goat farm in the region of Tübingen, Baden-Württemberg, Germany, had been reported. For confirmation oft he

outbreak goats were bled and serologically tested for TBE virus antibodies. Ticks were sampled around the goat stable and tested for TBE virus by real time PCR. A retrospective outbreak examination was conducted by the local public health office to identify potential consumers of the involved raw milk products and exposed persons were tested serologically for TBE antibodies.

Results: The TBE focus was confirmed by isolation of the TBE virus from ticks and TBE infection could be confirmed serologically in one of the five flock goats. The investigations of the local public health office identified 27 consumers of goat milk at the putative period of exposure. For 20/27 exposed persons, anamnestic information was gained by the local public health office. Twelve of 14 exposed and non-vaccinated people developed clinical illness and were confirmed as TBE cases by serology. Five/six vaccinated and exposed people did not develop the disease. The one exposed and vaccinated person had the last TBE vaccination booster more than 15 years ago, and therefore the booster was more than 10 years overdue. None of the regularly vaccinated and exposed persons developed clinical overt TBE infection.

Conclusison: To our best knowledge protection against the alimentary route of TBE infection by the available TBE vaccines could be demonstrated fort he first time during this TBE virus outbreak.

DP5

Tick-borne Encephalitis in Germany in the Times of COVID-19

Gerhard Dobler
Bundeswehr Institute of Microbiology, Virology and Rickettsiology, Munich, Germany

Introduction: Tick-borne encephalitis is the most important tick-borne virus infection in Europe and Asia. During the last years, a steady increase of registered human TBE cases in Germany has been observed. The reasons of this icnrease are unclear so far. During the year 2020 the COVID-19 pandemic restricted movements of German citizens. It was unclear, how these restrictions effected the incidence of TBE infections.

Methods: Human TBE case records from the epidemiological program of the Robert-Koch-Institute (RKI, SurvStat) were analysed according to their spatial and temporal appearance. The changes of TBE incidence in different parts of Germany were compared to changes of other infectious diseases (food-borne, aerogen, vector-borne) and tot he neighboring countries of Austria and Switzerland.

Results: In 2020 a total of 710 human TBE cases had been reported and registered in Germany by the RKI. This is the highest number ever recorded in Germany. A comparison with the neighboring countries showed that also in Austria and Switzerland record numbers were observed. The temporal analysis showed that there was a two-peak appearance of human TBE cases with the second peak probably induced by the COVID-19 situation. The spatial analysis showed that there was an dramatic increase of human cases in the districts with higher altitudes, mainly the Black Forest, the pre-Alpian Mountains areas, the German Central Low Mountain ranges and the Suebian Alb ranges. A comparison with other reportable infectious diseases showed that all other reportable infectious diseases decreased up to 70%, while TBE increased for more than 50% compared to the period of 2015 to 2019.

Conclusions: The analysis of human TBE numbers in 2020 showed some surprising features. These features seem not to be restricted to Germany but were also observed in Austria and Switzerland. Therefore, the highest ever recorded numbers of human TBE cases seem to have a multifactorial cause with effects of the hot weather (climate change?), human activities, but indirectly also by the COVID-19 situation.

DP6

Investigations into Middle East respiratory syndrome coronavirus in camels and humans in Sudan

Khalid A. Enan[1], Surtees R[2], Elhussein A[1], Mohamed O. Mustafa[3], Elkhidir I[1], Krause E[2], Hofmann N[2], Puyskens A[2], Nitsche A[2], and Michel J[2]
1- Central Laboratory- The Ministry of Higher Education and Scientific Research , Virology, Khartoum, Sudan; 2- Robert Koch Institute, Centre for Biological Threats and Special Pathogens, Division Highly Pathogenic Viruses (ZBS1), Seestraße 10, 13353 Berlin, Germany; 3- Central Laboratory- The Ministry of Higher Education and Scientific Research, Virology, Khartoum, Sudan

Introduction: The Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic human pathogen associated with severe respiratory symptoms and renal failure, with a fatality rate of up to 35 %. It is thought that MERS CoV is transmitted to humans from infected dromedaries, indeed MERS CoV has been detected in dromedaries in several countries. Human to human transmission of MERS CoV is possible, and has been reported in health care settings as well as more infrequently among family members.

Aim: We aimed to investigate the circulation of MERS-CoV in camels and humans in Sudan, in order to gain a better understanding of the risk of transmission to humans who live and work in close proximity to camels.

Methods: To this end, in 2016 - 2017 both nasal swabs (n=331) and serum samples (n=371) were collected from dromedaries in Kassala, Gezera, and Atbara while serum samples only (n=290) were collected from dromedaries in the River Nile and Khartoum states. Dromedary nasal swabs were then analysed for MERS CoV RNA by real time RT-PCR and camel serum samples for anti-MERS CoV IgG antibodies by ELISA. Human serum samples from healthy blood donors in Khartoum (n=92), and from patients presenting with atypical pneumonia in Khartoum (n=41) were analysed for anti MERS CoV IgG antibodies by ELISA, as were serum samples collected from people who worked in close contact with the dromedaries in Kassala (n=120), Gezera (n=92) and Alfaw (n=92).

Results: We report a high prevalence of MERS-CoV antibodies in dromedaries in several states ranging from 78 % in Khartoum to 96 % in Kassala State. MERS CoV RNA was detected in 1/234 nasal swabs (0.4 % prevalence) taken from dromedaries in Gezera and in 10/37 nasal swabs (27 % prevalence) from dromedaries in Atbara.

In human samples an anti-MERS-CoV IgG seroprevalence rate of 1.1% was found in both people who worked in close contact with dromedaries in Alfaw and blood donors from Khartoum, however none of the human serum samples from Kassala or Gezera were seropositive. In addition 2/41 patients (5 %) with atypical pneumonia were found to be seropositive for anti-MERS CoV IgG antibodies.

Conclusions: This study confirms the circulation of MERS-CoV in Sudan in dromedary populations. The discovery of human serum samples positive for MERS CoV IgG antibodies indicates the possible transmission of MERS CoV from camels to humans in Sudan.

DP7

Screening of ticks and mosquitoes collected in Sudan reveals the presence of multiple Phleboviruses, as well as Crimean-Congo hemorrhagic fever virus in ticks

Khalid Enan[1], Rebecca A. Surtees[2], Abdel El Hussein[1], Annika Brinkmann[2], Eva Krause [2], Natalie Hofmann[2], Andreas Puyskens [2], Janine Michel[2], Peter Hagedorn [2], Mohamed O. Mustafa[1], Isam Elkhidir [1], and Andreas Nitsche[2]
1- Central Laboratory, Virology department, Khartoum, Sudan; 2- Robert Koch Institute, Centre for Biological Threats and Special Pathogens, Division Highly Pathogenic Viruses (ZBS1), Berlin, Germany

Several highly pathogenic viruses are transmitted to humans through the bite of infected ticks or mosquitoes. These include (but are not limited to) the tick transmitted Crimean-Congo hemorrhagic fever virus (CCHFV), a member of the *Nairoviridae* family, and the mosquito transmitted Rift Valley fever virus (RVFV), a member of the *Phenuiviridae* family, genus *Phlebovirus*. CCHFV is primarily a human pathogen with a case fatality rate of up to 50 % that circulates asymptomatically in its arthropod and animal hosts. RVFV on the other hand is a pathogen of both humans and animals, causing devasting "abortion storms" in infected ruminants and Rift Valley fever disease in humans. Outbreaks of both CCHFV and RVFV have been documented in Sudan. In addition to these known highly pathogenic viruses, many other viruses replicate in ticks and mosquitoes, whose ability to infect and cause disease in humans and animals is unknown. This study aimed to investigate the prevalence of Phleboviruses (including RVFV) in ticks and mosquitoes in Sudan as well as the presence of CCHFV in ticks. Ticks and mosquitoes were collected from 4 states across Sudan between 2016 and 2018 and analysed using a generic pan-Phlebovirus RT-PCR assay and a CCHFV or RVFV specific RT-qPCR assay. Overall 30/56 (53.6 %) of the analysed ticks pools and 19/212 (8.96 %) of the analysed mosquito pools were Phlebovirus positive. PCR amplification products were sequenced revealing that sequences from 12/19 Phlebovirus positive mosquito pools shared a high similarity with Phasi Charoen-like virus, and sequences from 28/30 Phlebovirus positive tick pools shared high similarity with Phlebovirus sequences previously detected in ticks in Turkey. No mosquito pools were positive for RVFV. One pool of ticks collected from a camel in Port Sudan was positive for CCHFV RNA; partial sequencing of the L-segment revealed the CCHFV sequence to be highly similar to other CCHFV strains documented in Sudan in 2008, as well as a strain from Namibia. The discovery of CCHFV in ticks taken from a domestic camel highlights the potential threat posed by this highly pathogenic virus to the camel herders that live and work in close proximity to their herds. The presence of Phleboviruses of unknown pathogenicity in such a high proportion of ticks and mosquitoes in Sudan was an unexpected finding, and their zoonotic potential bears further investigation.

DP8

Longitudinal Survey of West Nile Virus Infection in Horses in Chad (Central Region)

Thomas Normand[1], Gilda Grard[2], Olivier Carette[3], Tawfik A. Razakh[4], Cédric Roqueplo[5], Jean-lou Marié[6], Isabelle Leparc-Goffart[2], and Bernard Davoust[7]

1- 12ème Centre Médical des Armées, 41ème Groupe Vétérinaire, Fontainebleau, France; 2- IRBA-Institut de Recherche Biomédicale des Armées, French National Reference Centre for Arboviruses, Marseille, France; 3- 1er Centre Médical des Armées, 23ème Groupe vétérinaire, Paris, France; 4- Veterinary Service of the National and Nomadic Guard of Chad, N'Djamena, Chad; 5- Animal Epidemiology Expert Group of the Military Health Service, Tours, France; 6- French Military Health Service, Expertise and Defense Health Strategy Division, Paris, France; 7- IHU Méditerranée Infection, Marseille, France

Serological evidence of the West Nile virus (WNV) infection was sought in 2012 and 2016 on a horse population of the National and Nomadic Guard of Chad (GNNT). Each sample was tested for IgG against WNV by using an in-house ELISA with precipitated and inactivated WNV as antigen. Microneutralization tests against West-Nile and Usutu viruses were used to confirm the specificity of the IgG-positive and doubtful sera. In total, 69.7% (62/89) of the horses collected in 2012 were seropositive or doubtful compared to 91.5% (54/59) in 2016. A cohort of 39 of these horses was collected in both 2012 and 2016. Twelve from these 39 horses were seronegative in 2012 but only one in 2016. The rate of 91.7% (11/12) of seroconversion show an active circulation of flaviviruses in this sub-Saharan region. The West-Nile infection was confirmed by seroneutralization tests for nine of these samples. The low mobility and turnover observed in horses of GNNT show that positive animals had been infected close to the sampling sites. This is important if trying to identify environmental features associated with the seroprevalence rate. The demonstration of a significant infection of equids in Chad highlights the persistence in Africa of a viral enzootic cycle between migratory birds and mosquitoes (circulation and viral amplification) but with a spatial distribution that remains difficult to pinpoint. The IgG antibodies can persist for several years in horses, but the seroconversion rate observed in this study confirms that flaviviruses and therefore most likely WNV do actively circulate in Chad. Some other flaviviruses may also be transmitted to equids as suggested by samples that are ELISA positive and neutralization negative. The cycling process of virus between mosquitoes and birds provides a reservoir for the virus that may then be transmitted to dead-end hosts such as humans and horses. Therefore, surveillance offers one option for monitoring the emergence of disease in human populations. It has been advocated that horses could be used as sentinels for detecting West Nile virus activity and emerging viral strains.

DP9

Tula Orthohantavirus (TULV) as the predominant species in three regions in Kazakhstan

Nur Tukhanova[1,2]

1- Center for International Health, University Hospital, LMU Munich, Germany; 2- Central Reference Laboratory, NSCEDI, Almaty, Kazakhstan

N. Tukhanova extsuperscript1,2, A. Shin extsuperscript1,2, N. Turebekov extsuperscript2, T. Nurmakhanov extsuperscript2, A. Shevtsov extsuperscript3, K. Abdiyeva extsuperscript4, V. Sutyagin extsuperscript5, A. Berdibekov extsuperscript5, N. Maikanov extsuperscript5, A. Andruyshenko extsuperscript5, L. Yeraliyeva extsuperscript6, G. Froeschl extsuperscript1,7, M. Hoelscher extsuperscript7, E. Wagner extsuperscript8, L. Peintner extsuperscript8, S. Essbauer extsuperscript8

extsuperscript1Center for International Health, University Hospital, LMU Munich, Germany extsuperscript2Central Reference Laboratory, NSCEDI, Almaty, Kazakhstan extsuperscript3National Center for Biotechnology, Nursultan, Kazakhstan extsuperscript4Branch National Center for Biotechnology, Almaty, Kazakhstan extsuperscript5NSCEDI Antiplague Station, Kazakhstan extsuperscript6National Scientific Center of Phthisiopulmonology, Almaty, Kazakhstan extsuperscript7Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich, Germany extsuperscript4 extsuperscript8Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, Munich, Germany

Orthohantaviruses (OHV) are zoonotic pathogens that play a significant role in public health. Several small mammals are reservoirs of OHV and can cause hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) in humans. The first human case of HFRS was registered in West Kazakhstan region in the year 2000. A following investigation into small mammals in West Kazakhstan showed antigens for OHV in wild living rodents in four districts of West Kazakhstan region and proved a natural reservoir for OHV. So far, molecular genetic data on OHV in Kazakhstan are limited. The aim of this study is to investigate small mammals for the presence OHV by molecular

methods and to provide a molecular characterization of the circulating strains in Kazakhstan.

The study was set up in 2018-19. Small mammals were trapped in West Kazakhstan region, Almaty region and Almaty city throughout the year.

In total 621 small mammals from 11 species were investigated. Among the collected small mammals 2.4% tested positive for orthohantavirus S segment. The prevalence of positive samples for OHV was 0.4% in West Kazakhstan and 7% in Almaty region respectively. Sequencing parts of the S segment specified Tula OHV (TULV) as the predominant virus in these two regions.

Our data showed that geographical distribution of TULV OHV is more extended as previously thought. Two species of rodents (*Microtus arvalis* and *Dryomys nitedula*) were positive for TULV in Kazakhstan. It needs further investigation of host reservoirs among the country to understand the full distribution of OHV in Kazakhstan.

EP1

The unique cases of two Spanish isolates of *Bacillus anthracis* within the Trans-Eurasian clade A.Br.008/009

María V. Ortega[1], Olga Bassy[1], Markus Antwerpen[2], Wolfgang Beyer[3], Javier Del olmo[1], Juan C. Cabria[1], Roman Wölfel[2], and Gregor Grass[2]
1- INTA, CBRN, San Martín de la Vega, Spain; 2- Bundeswehr Institute of Microbiology, Munich, Germany; 3- University of Hohenheim, Stuttgart, Germany

Within the major lineage A, in the A.Br.008/009 canonical single-nucleotide polymorphisms (canSNP) group of *Bacillus anthracis*, also known as the Trans-Eurasian group (TEA), two Spanish new sub-clades were recently established by whole genome SNP analysis (wgSNP). The TEA is one of the dominant groups of *B. anthracis* in the world with isolates from countries in Europe, Russia, Kazakhstan, the Caucasus region and the People's Republic of China, including a few African and American strains.

More than ten mostly minor distinct sub-clades related to the clade A.Br.008/009 have been recently defined. Among these group two Spanish isolates with unique characteristics. One Spanish strain 319/02 branched off the A.Br.159 polytomy (separated by 379 apomorphous SNPs), whereas strain 342/02 branched off directly from the A.Br.011 polytomy (separated by 107 SNPs). Compared to typically short branch-lengths of the TEA clade, both Spanish isolates displayed characteristically long branches. This may be due either to an extended evolutionary history or nucleotide-changes caused by an increased mutation rate. Especially, isolate 319/02 may possibly be a mutator strain as evidenced by an enormous number of phylogenetically uninformative SNP-positions. This strain shared two basal SNPs with canSNP group A.Br.147, the clade leading to A.Br.WNA sub-clade, distributed across North America. Strain 319/02 also shared four synapomorphus SNPs with strains Gmb1 (The Gambia), Sen2Col2 and Sen3 (Senegal), hence this Spanish strain was the closest European isolate to these African strains (632 SNPs between strains 319/02 and Sen2Col2). Conversely, the closest relatives of strain 342/02 were the French strains ANSES_088, A169 and A193a/A0401. Notably, the genetically very different Spanish isolates 319/02 and 342/02 differed only by one 31 variable number tandem-repeat (MLVA) markers highlighting the superior phylogenetic resolution power of genomic SNP-based analysis. Our results suggest that Spain harbours a *B. anthracis* population that may constitute a phylogenetic bridge between Europe, North America and possible Africa. Additional isolates from the Iberian Peninsula, its neighbouring countries in Western Europe and North-Western Africa, North America, and France are needed to test this hypothesis.

EP2

Metagenomics for biodefense

Mehdi Abdelli[1], Xavier Tichadou[1], Maylis Dadvisard[1], Vincent Ramisse[1], David Christiany[2], Jean-philippe Vernadet[2], Gilles Vergnaud[2], Anna Anselmo[3], Giovanni Faggioni[3], Florigio Lista[3], Andreas Sjödin[4], Edvin Karlsson[4], David Sundell[4], Mats Forsman[4], Flavia Dematheis[5], Mathias Walter[5], and Markus Antwerpen[5]
1- DGA CBRN Defence, Vert-le-Petit, France; 2- Université Paris-Saclay, Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France; 3- Army Medical Center, Scientific Department, Roma, Italy; 4- Swedish Defence Research Agency, CBRN Defence and Security, Umeå, Sweden; 5- Bundeswehr Institute of Microbiology, Munich, Germany

Metagenomics refers to the study of total genetic material from a mixed community of organisms and has increasing popularity for deciphering the microbial diversity of environmental biosamples. Advances in both sample preparation and high throughput DNA sequencing have opened the way for metagenomic detection of pathogens of interest without excluding those unknown to date. It is likely an interesting approach to complement

the ultrasensitive PCR, which is the current gold standard for biodetection. PCR suffers some drawbacks, it is poorly multiplexable, limited to targeted pathogens and blind to the presence of unknown ones. Importantly, false positives are difficult to deal with.

The search for pathogens in a biological sample by analysis of global genetic material is simple in principle and several bioinformatic tools allow to inventory a mass of genetic data. Recent tools based on k-mer approaches use reference genomes and a taxonomy to assign each segment of the sequence to a taxonomic level.

However two major challenges must be addressed. Firstly, the produced inventories may be inaccurate (specificity). For example, some studies have erroneously concluded that major pathogens such as *Bacillus anthracis* are present in some urban environments. In this case, the misinterpretation was mainly due to the inadequacy of the method used, i.e. the lack of a robust phylogenetic approach allowing the positioning of sequence data at subspecies level. This problem made it difficult to distinguish between *B. anthracis* and its closest relatives. Secondly, the sensitivity of the metagenomics approach might be too low for practical purposes in the presence of a rich biological background.

To address the issue of specificity, a pipeline B2forensics_v1 was developed, based upon the *Kraken2* software complemented by additional filters which allow to precisely evaluate the phylogenetic position of candidate reads within the *B. anthracis* population. To address the issue of sensitivity, we have developed a realistic benchmark data set by sequencing DNA extracted from waste-water. Prior to DNA extraction and sequencing, half of the samples were spiked with real biological agents or with close neighbours. We then evaluated the performance of B2forensics_v1 alongside similar published tools. The pipelines were compared using the benchmark dataset. We will present the results we have obtained so far with a focus on *B. anthracis*.

EP3

Machine learning algorithms for classification of MALDI-TOF MS spectra from phylogenetically closely related species like *Brucella melitensis*, *Brucella abortus* and *Brucella suis*

Flavia Dematheis[1], Mathias C. Walter[1], Holger C. Scholz[2], Markus Antwerpen[1], Marie T. Pfalzgraf[1], Christin Hinz[1], Roman Woelfel[1], and Sabine Zange[1]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- Robert Koch Institut, Berlin, Germany

MALDI-TOF mass spectrometry is a reproducible and reliable tool for microbial fingerprint-based characterization. However, for phylogenetically closely related species such *B. melitensis*, *B. abortus* and *B. suis*, the resolution power drops down to the genus level.

The major aim of this study was to analyze a collection of 44 clinical isolates of *Brucella*, including 20 *B. melitensis*, 13 *B. suis* and 11 *B. abortus* in order to: (i) identify the most appropriate classification methods among the most popular supervised and unsupervised machine learning algorithms; (ii) discover marker peptides for *Brucella* spp. to get further insight into biological processes related to the different species.

A consensus feature selection method was applied to pinpoint variables to feed ML algorithms. Unsupervised learning algorithms such as k-means and Hierarchical Agglomerative Clustering (HAC) were applied by means of the R-packages "cluster" and "Caret". Models with a different number of cluster centers were computed and evaluated using the silhouette coefficient (SC). For HAC, different distance metrics and different linkage functions were explored as well using *dist* and *hclust* R-functions. Supervised models such as Random Forest, Neural Network, Support vector machine, Logistic regression and Linear discriminant analysis were computed using the corresponding R-packages "randomForest", "nnet", "e1071", "glmnet" and "sda". Fine-tuned models were trained using "Caret" upon definition of a model specific hyperparameter grid. Model performance was assessed using a nested k-fold cross validation (CV) method with a feature selection step between the two CV loops to avoid overfitting. Overall accuracy was used as a matrix to estimate the performance of the learning systems.

The unsupervised HAC over-performed the k-means algorithm and allowed an accurate label-independent species recognition. All fine-tuned supervised methods exhibited a predictive performance with an accuracy ranging between 96.4 and 100%. The most discriminative peaks among *Brucella* sp. were identified. However, the identity of the corresponding peptides could not be determined by the use of the ExPASy Bioinformatics resource portal, when the PeptIdent tool was applied to match the molecular weight with the UniProtKB/Swiss-Prot database.

EP4

Case report and epidemiological analysis of an anthrax outbreak occurred in Calabria region (Italy) involving three human cases

Domenico Galante[1], Luigina Serrecchia[1], Giuseppe

Lucifora[2], Maurizio Guastalegname[3], Viviana Manzulli[1], Lorenzo Pace[1], Dora Cipolletta[1], Antonio Parisi[4], Angelica Bianco[4], Loredana Capozzi[4], Fiorenza Petruzzi[1], Ines Della rovere[1], and Valeria Rondinone[1]
1- Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy; 2- Istituto Zooprofilattico Sperimentale del Mezzogiorno, Vibo Valentia, Italy; 3- Ospedale Jazzolino , Unità di Malattie Infettive, Vibo Valentia, Italy; 4- Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Putignano, Italy

Anthrax, caused by the bacterium *Bacillus anthracis*, is a disease with a natural transmission cycle involving wildlife, livestock, and, occasionally, humans. Domestic and wild ruminants are the species more susceptible to anthrax.

In Southern Italy, animal anthrax is an endemic disease with sporadic episodes occurring usually during hot dry summers preceded by rainy springs. A recent outbreak in Italy occurred in Calabria region in 2020, caused by a bullock suddenly dead in a farm located in the province of Vibo Valentia, without the typical symptoms of anthrax, and involving two farmers and one butcher who came in contact with the animal and later all of them showed a severe cutaneous form. Moreover 2 out of the 3 people also complained of a strong headache. In one the patient was collected liquor by lumbar puncture because anthrax meningitis was suspected. The three people were immediately treated with ciprofloxacin, penicillin, meropenem and steroids.

Bacillus anthracis was isolated from 3 samples of the bullock, from muscle, prescapular lymph node and drainage blood and later confirmed by PCR. *B. anthracis* detection was not possible from cutaneous eschars in the 3 patients because of the antibiotic treatments, but just from liquor, confirming the presence of meningitis.

Phylogenetic analysis was determined through the research of polymorphisms for CanSNPs, with 14 PCR assays for allelic discrimination. A 31-loci MLVA assay was performed to determine *B. anthracis* genotypes.

The analysis of 14 CanSNPs, collocated the strain into the major lineage A, sublineage A.Br.008/011, differently from a *B. anthracis* strain responsible of an anthrax outbreak that occurred in the same region in 2002, in the province of Cosenza. This last one belongs to the major lineage A, sublineage A.Br.011/009, which is the most widespread in Italy.

Also the strains isolated in Umbria region in 2009 and several strains isolated in Sicily region during the anthrax outbreaks occurred in the last fifteen years in Italy belong to the sublineage A.Br.008/011. The MLVA with 31 VNTRs analysis demonstrated a new genotype, different from the previous detected in Calabria in 2002. It showed differences in four loci.

In conclusion, no genetic correlation has been evidenced between *B. anthracis* strains isolated in Calabria in 2020 and 2002.

The three infected people healed some days after the treatment with different antibiotics.

EP5

Close genetic relationship between *Klebsiella pneumoniae* from urinary tract infections (UTIs) and retail meat recovered in Spain during 2020

Isidro García-Meniño[1], Luz Lestón[2], Raquel Fernández-pedrero[2], Pilar Lumbreras[3], Javier Fernández[3], Annemarie Kaesbohrer[1], Azucena Mora[4], and Jens A. Hammerl[1]
1- German Federal institute for Risk Assessment, Biological Safety, Berlin, Germany; 2- Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Departamento de Microbioloxía e Parasitoloxía, Lugo, Spain; 3- Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Grupo de Microbiología Traslacional, Oviedo, Spain; 4- Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Departamento de Microbioloxía e Parasitoloxía, Spain

Background: *Klebsiella pneumoniae* is recognized as a major cause of nosocomial infections and one of the predominant bacteria associated with urinary tract infections (UTIs) in both community and health care settings, worldwide. Besides their clinical impact, there is a gap of knowledge on the impact of retail meat products in the spread of globally emerging *K. pneumoniae* high-risk clones. This study aims the genetic and genome-based comparison of *K. pneumoniae* isolates recovered from human infections and retail meat products of the same geographical area in Spain under the "One Health" approach.

Materials and Methods: In 2020, 64 clinical *K. pneumoniae* isolates were consecutively recovered from human UTIs (n=52) and retail meat products (turkey meat, n=8; chicken meat, n=3 and pork meat, n=1) in Oviedo (northwest of Spain). *In depth* characterization including whole-genome sequencing (WGS) was performed for all isolates. The phylogenetic relationship of the *K. pneumoniae* was analysed using XbaI-macrorestriction (XbaI-PFGE) profiling as well as MLST and SNP analysis of the core genomic regions.

Results: The comparison of the XbaI-PFGE profiles revealed a high diversity within the collection. A total of seven phylogenetic clusters were detected. Four of them contained both meat- and human-associated isolates, which appeared scattered throughout the PFGE dendrogram. It is of note that two turkey meat isolates were assigned to the recently emerging high-risk clones ST307 and ST147, respectively. Both of them exhibited high-identity XbaI-macrorestriction profiles (>85%) with two human isolates. Three further sequence types previously detected in clinical cases (ST111, ST983 and ST219) were also identified among the food strains. Finally, SNP-based phylogenetic analyses confirmed the relationship between meat- and human-associated isolates.

Conclusions: This "One Health" approach indicates a high genetic identity between meat *K. pneumoniae* isolates of meat and human clinic (UTI) origin in the same geographical region. In view of our results, poultry meat products would represent a yet underestimated source of human clinically important *K. pneumoniae* clones. To prevent a further spread of high-risk *K. pneumoniae* clones in the future, a better understanding of the zoonotic impact of these bacteria and novel public health surveillance strategies are needed.

EP6

Genetic characterization of *Bacillus anthracis* isolates by use of Whole Genome Sequencing data

Angelica Bianco, Loredana Capozzi, Laura Del sambro, Domenico Simone, Luigina Serrecchia, Viviana Manzulli, Valeria Rondinone, Antonio Fasanella, Antonio Parisi, and Domenico Galante
Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy

Bacillus anthracis (*B. anthracis*) is a member of the *B. cereus* group, gram positive spore forming soil bacteria. Despite their close genetic correlation with other bacteria of this group, the *B. anthracis* species exhibit certain specie-specific phenotypes, some of which are related to pathogenicity. *B. anthracis* is known to be responsible to anthrax, a fearsome zoonotic disease. Vegetative cells of *B. anthracis* can produce anthrax toxin proteins and capsule, which are encoded by pXO1 and pXO2 plasmids, respectively. We collected fifty-seven *B. anthracis* strains isolated in Italy during different anthrax outbreaks between 1984 and 2018. The entire DNA was purified from each strain and whole genome sequencing (WGS) was performed. Genetic characterization was based on species identification by ANIBlast, Sequence Type and phylogenetic groups definition and major virulence factors and antimicrobial genes (AMR) identification. All strains were predicted *as B. anthracis* belonging to *B. mosaicus* genomospecies with ANI score value >99.9% and, based on the *panC* sequence analysis, all strains match with the phylogenetic group III. The 98% of strains belonged to ST1, whereas the 2% to ST3. The typical anthrax toxins were encoded by the genes *lef*, *pag* and *cya*, that map onto two plasmids pXO1 and pXO2. We identified the co-presence of these genes in the 77% of strains. Additionally, we identified the gene cluster NHE, the cluster gene of *capABCDE*, *sph* and *bpsE* in all strains analysed; the cluster gene of *HasA-C* was identified in 82% of strains. Interestingly, for the strains that did not harboured the three genes *HasA-C*, we did not identify the three genes encoding the typical anthrax toxins. Based on AMR genes predicted, all strains might show resistance to beta-lactamase, clindamycin, fosfomycin and glycopeptide antibiotic. Taken together, our finding showed that the 77% of strains analysed were potential virulent, based on the presence of the two plasmid pXO1 and pXO2. Additionally, the analyses carried out allowed additional virulence factors typical of members belonging to the *B. cereus* group, that might increase the pathogenic role of these bacteria. Although the predicted antimicrobial resistance would need phenotypic validation in order to address antibiotic treatment, the approach based on WGS is a robust method to define the rapidly genetic characterization of a large number of potential pathogen bacteria.

EP7

SARS-CoV-2 clusters identification and characterization: transmission dynamics at the Italian Army Medical Center

Rossella Brandi[1], Anella Monte[1], Marzia Cavalli[1], Lucia Nicosia[1], Giulia Campoli[1], Antonella Fortunato[1], Anna Anselmo[1], Francesco Giordani[1], Vanessa V. Fain[1], Pietro M. d'Angelo[2], Riccardo De Santis[1], Giovanni Faggioni[1], Valeria Franchini[1], Giandomenico Cerreto[1], Alfonso Giordano[1], Florigio Lista[1], and Silvia Fillo[1]
1- Army Medical Center, Scientific Department, Rome, Italy; 2- CNR, IASI, Rome, Italy

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) originated in Wuhan (China) in early December 2019 and rapidly spread worldwide. Over the course of the pandemic SARS-CoV2 genome sequencing allowed valuable insights into the ongoing evolution, epidemiology, identification of circulating genetic variants. The molecular surveillance of SARS-CoV2 has proved crucial for monitoring

the spread of emerging mutations and 'variants of concern' (VOCs) and/or "variants of interest" (VOIs).

From the beginning of the pandemic, the Scientific Department of the Army Medical Center in Rome (AMC), employed many different Next Generation Sequencing (NGS) approaches (Illumina, MGI and Oxford Nanopore technologies) to investigate Sars-CoV2 genome features.

With the emergence of the Alpha Variant (B.1.1.7) the Italian Ministry of Health established a surveillance system based on the sequencing of SARS-CoV2 positive samples. Thus, all Italian regional reference laboratories started an intensive genomic analysis. In addition, the Scientific Department of the AMC organized a network of 12 military laboratories (DIMOS_MILNET) distributed across the country that collected swabs and sent positive samples to the reference center for subsequent sequencing.

The aim of this study is to analyze SARS-CoV-2 genomes to investigate the genetic diversity of SARS-CoV-2 strains spread in our country during the different pandemic waves.

From the first reported case of an Alpha variant in Italy (belonging to a traveler from the UK and identified on December 14th by our laboratory) to late January 2021, 24.5% of our cases were Alpha Variant. This percentage increased up to 91,3% at the end of April 2021 and then dropped to 14.2% in June. The decrease in Alpha VOCs was inversely proportional to the increase in Delta cases (B.1.617.2) as we registered 0,3% of Delta VOCs in April 2021, 21% in May and 74% in June. As regards Beta (B.1.351) and Gamma (P.1) VOCs, we observed only a few cases never reaching a percentage higher than 10%. The development of large-scale sequencing networks worldwide may level-up the molecular surveillance of SARS-CoV2 evolution and the process of clinical risk stratification. To this aim, the generation of broad datasets including clinical data, age, gender, social origin, previous infection or vaccination and belonging geographical area, may also pave the way for a deeper understanding and a stronger response to current and future pandemics.

EP8

Profiling the *Coxiella burnetii* resistome with a neural network based on amino acid composition of Position-Specific Scoring Matrix (PSSM) profiles

Akinyemi M. Fasemore[1], Andrea Helbich[1], Mathias C. Walter[1], Thomas Dandekar[2], Konrad Förstner[3], and Dimitrios Frangoulidis[4]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- University of Wuerzburg, Bioinformatics, Wuerzburg, Germany; 3- ZB MED Information center for life sciences, Data Science and Services, Cologne, Germany; 4- Bundeswehr Medical Service Headquarters VI-2 Medical Intelligence & Information (MI2), Munich, Germany

Coxiella burnetii is the aetiological agent of Q fever, a zoonotic disease that affects predominantly small ruminants and humans. Therapy of acute and chronic disease is based on doxycycline. Resistance problems in therapy are seldom but have been described in chronic Q fever. Anti-microbial sensitivity testing in *C. burnetii* is difficult due to the strict intracellular growth of the pathogen. Therefore, information about anti-microbial resistance is very limited. In general, discovering all antibiotic resistance genes (ARGs) of a pathogen (resistome) is an important approach to support and improve therapy of infectious diseases.

The "best hits" approach on sequence level is the most common method for resistome profiling, however this approach has not been successful with *Coxiella* genomes. To this end, we developed a machine learning (ML) approach that is based on artificial neural network, while leveraging the availability of ARGs databases to enhance analytic power, prediction quality and accuracy. Our implementation employed a feature characterization method that is based on amino acid composition of PSSM profiles to encode protein sequences. The evaluation of our model with novel and known ARG sequences showed ≈ 0.96 accuracy as well as high precision and recall.

We applied the model to predict ARGs from 61 *C. burnetii* genomes that were downloaded from RefSeq database. We observed that the most predicted ARGs in *C. burnetii* belong to the multidrug category, followed by macrolides-lincosamide streptogramin (MLS) and beta lactamase. Predicted multidrug ARGs were majorly efflux proteins with known transmembrane multidrug activity. The beta lactam ARGs were known lactamase with pseudo sequences and the predicted MLS were mainly proteins with ATP binding activities. The former could be an explanation for the well-known inefficiency of beta lactam antibiotics in Q fever therapy, whereas the latter is somehow unexpected, because macrolide antibiotics are used as second line antibiotics in acute Q fever.

Our study is the first to identify ARGs in *Coxiella* using a ML approach on amino acid level. This work should contribute to the body of information about antibiotic resistance properties in *C. burnetii* genomes and should be considered in future genome analyses.

EP9

Real-life experience from >1 year of mNGS pathogen identification with DISQVER

Silke Grumaz, Sandra Unsleber, Petra Horvatek, and Philip Stevens
Noscendo GmbH, Duisburg, Germany

Metagenomic Next-Generation Sequencing (mNGS) for the identification of pathogens from blood samples based on circulating, cell-free DNA as previously published (Grumaz et al. Genome Med 2016, Grumaz et al. Crit Care Med 2019) is now commercially available in Germany and Austria as a send-out service (DISQVER® CE-IVD test by Noscendo GmbH). mNGS provides crucial advantages in comparison to conventional testing: it more frequently provides a positive result than blood culture (BC), it requires no a priori knowledge of the causative pathogen and several organisms including dsDNA viruses, bacteria, fungi or parasites can be identified in one test, even when antimicrobial treatment has already been initiated. We here describe the experience of over 1 year of send-out service and highlight a few select cases. Data was collected by evaluating the mNGS data and questionnaires accompanying the send-out sample. DISQVER® blood test was most frequently ordered for sepsis (35%), hematology (33.9%), Covid-19 (19.3%), cardiology (9.3%) and following transplantation (2.5%). Positivity rate was 50.7 % (sepsis), 50.5% (hematology), 40.7% (Covid-19) and 32.8% (cardiology). Of those positive results, in most cases only one pathogen was reported (54.1%), followed by two (20.4%) or three (10.8%) pathogens. More than 4 pathogens were reported in 14.8% of positive results, combined. In positive results over all indications, bacteria were most frequently identified (50.2%), followed by dsDNA viruses (39.6%), fungi (9.3%) and parasites (0.8%). The advantages of untargeted mNGS pathogen identification become especially evident for rare and/or fastidious organisms, which can be difficult to detect with standard methods. Select examples from the observation period include a patient with severe and persistent Aspergillus fumigatus infection following heart transplantation, with confirmatory cultivation-based results several weeks after positive DISQVER® results. Further cases were Pneumocystis jirovecii pneumonia in a HIV-positive patient, a case of Coxiella burnettii endocarditis and several cases of mucormycoses (Rhizomucor) in immunocompromised patients which were all initially not identified with standard methods but could be confirmed afterwards. In conclusion, 1 year of send-out service for mNGS provided clinicians with a high number of clinically relevant, positive results, in many cases with unexpected pathogens and frequently in absence of results from standard diagnostics.

EP10

Comparison of sporulation in *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis*

Constanze Gummelt[1], Susann Dupke[1], Michael Laue[2], Sabine Howaldt[1], Holger Scholz[1], and Silke R. Klee[1]
1- Robert Koch-Institut, Centre for Biological Threats and Special Pathogens (ZBS2), Berlin, Germany; 2- Robert Koch-Institut, Centre for Biological Threats and Special Pathogens (ZBS4), Berlin, Germany

Sporulation in bacilli is a strictly regulated process involving a sequence of sigma factors either active in the mother cell or in the fore spore. In *B. cereus* biovar *anthracis* (*Bcbva*), the gene *sigK* for the late sigma factor SigmaK is interrupted by a 22 kb insertion which is excised in the mother cell during sporulation. This resembles the situation in *B. subtilis*, but is very rare for the *B. cereus* group. In an attempt to compare gene regulation during sporulation, we analyzed two strains of *Bcbva* and two strains of *B. anthracis*. Bacteria were grown in sporulation medium, and RNA was extracted at the onset of stationary phase, one hour and two hours later. A panel of sporulation genes, including some encoding sigma factors, spore coat proteins or a small acid soluble protein, was chosen for expression analysis. We performed relative quantitation by qPCR with normalization to a housekeeping gene. Our analyses were complemented by electron microscopy studies of sporulation stages up to 24 hours of growth, which did not show any differences between *B. anthracis* and *Bcbva*. The overall gene expression profiles of the four strains were similar, and sporulation of *Bcbva* was not compromised by excision of the *sigK* gene - in contrast, sporulation was initiated earlier and might reflect a faster reaction of the bacteria to nutrient limiting conditions. Two spontaneous mutants of *Bcbva* defective in sporulation were also analyzed, in which expression of sporulation genes was almost undetectable, and no signs of sporulation were observed after 24 hours of growth. One mutant possessing a frameshift in the *spo0A* gene (nucleotide insertion resulting in premature stop codon) was able to revert to the wildtype phenotype after spontaneous deletion of a nucleotide upstream the insertion, which resulted in one amino acid exchange only.

EP11

Occurrence, properties and diversity of Klebsiella-specific phages from fecal sam-

ples of German wildlife and their suitability for therapeutic application

Claudia Jaeckel, Silvia Schmoger, Ashisch Gadicherla, and Jens A. Hammerl
German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

Questions: *Klebsiella pneumoniae* are important bacteria that frequently arise in clinical settings causing severe nosocomial infection. Due to their potential to efficiently exchange genetic material with other organisms, these bacteria force their adaption to prevailing environmental conditions. In the last decades, the number of multidrug-resistant *K. pneumoniae* in clinical settings had increased significantly, leading to a potential limitation of the therapeutical options in the human medicine in the future. Due to the necessity of alternative strategies for the treatment of *K. pneumoniae* infections in human, the suitability of lytic phages is actually studied worldwide.

Methods: The occurrence of phages in feces samples of wildlife hunted in Germany between 2018 and 2020 was determined by phage activity tests. The recovered phages were characterized in their phenotypic (host rang, plaque formation, TEM) and genotypic properties (WGS) to determine their potential for the treatment of *Klebsiella* spp. bacteria.

Results: The occurrence and characteristics of *Klebsiella*-specific phages in fecal samples from game was investigated to provide the community potential novel phage-prototypes for biocontrol and therapeutical issues. However, the number of recovered phages from fecal samples of the analysed game seemed to be low. By investigating more than 200 samples, only a few *K. pneumoniae*-specific phages could by recovered from wildlife feces of animals hunted in Brandenburg (Germany). The recovered phages exhibit rather narrow host ranges that are limited to very specific *K. pneumoniae* isolates. WGS revealed that the phages belong to different families of both temperate and lytic genera. Furthermore, sequence analyses indicated that independent from their lifestyles the recovered phages did not carry antimicrobial resistance and/or virulence determinants. Further information on phenotypic and genotypic properties of the phages will be presented.

Conclusions: On the basis of the prevailing results, feces samples of wildlife did not represent a common source for lytic phages against *K. pneumoniae*.

EP12

High-Throughput sequencing, variant detection and automatic report generation from diagnostic samples during the SARS-COV-2 pandemic

Maximilian Klepsch, Malena S. Bestehornwillmann, Mike H. Pillukat, Alexandra Rehn, Josua Zinner, Peter Molkenthin, Mathias C. Walter, and Markus Antwerpen
Bundeswehr Institute of Microbiology, Munich, Germany

During the Covid-19 pandemic the need for rapid sampling and fast assessment of SARS-COV-2 variants, especially since variants of concern emerged, is crucial for military personnel, considering international assignments. Foreign assigned staff bear the risk to function as vectors between country of deployment and origin. Therefore, we developed a workflow to standardize sequencing, data analysis and report generation for Covid-19 samples.

Next-generation library preparation is routinely performed at the Bundeswehr Institute of Microbiology using an enrichment protocol for SARS-CoV-2 and sequencing is performed using an Illumina MiSeq instrument. Two in-house pipelines are used to analyze and assess the Covid-19 sequencing data. The first pipeline is a multipurpose next-generation sequencing analysis pipeline for Illumina based-technologies. For the Coronavirus analysis, we map the sequencing data against the Wuhan-Hu-1 Reference genome (NC_045512.2). The resulting alignments are clipped and filtered prior to variant detection. The pipeline is also capable of analyzing amplicon-based protocols. Optionally taxonomically classification of raw reads can be performed. The second in-house pipeline was specifically written for the analysis of SARS-CoV-2. It is based on the results of the first pipeline and determines clade and lineage from the consensus sequences. The "Corona"-pipeline then creates a template with R markdown, which is used to finalize the report. Information about the quality, the coverage against the reference genome, detected mutations as well as associated lineages are included and visualized.

The presented modular approach enables rapid adaptation to constantly changing requirements during SARS-CoV-2 epidemics and can also be adapted for future outbreak analysis.

EP13

EuroThrax - Detection, Bioforensics and Genomics of *Bacillus anthracis* and highly pathogenic *Bacillus cereus* s.l.

Maximilian F. Mayerhofer[1], Gregor Grass[2], and Monika Ehling-Schulz[3]
1- Austrian Armed Forces, Armament and Defence Technology Agency, Vienna, Austria; 2- Institut

für Mikrbiologie der Bundeswehr, Sanitätsakademie der Bundeswehr, Munich, Germany; 3- Institut für Mikrobiologie , University of Veterinary Medicine, Vienna, Austria

Trace back analysis of biological agents is the prime directive of public authorities after initial medical first response. Detailed knowledge about the pathogen is of utmost importance to rapidly distinguish natural contamination from intentional contamination events in order to initiate appropriate measures. In an ever-growing interconnected world, a holistic approach on an international level is needed to combat outbreaks. This project focuses on Bacillus anthracis, the etiological agent of anthrax, which is a zoonotic spore forming bacterial pathogen that affects humans and animals. B. anthracis has gained increased prominence because of its past and recent misuse as an agent of bioterror. With the ongoing climate change the organism's habitability in European soils is possibly increasing and, as a consequence, the likelihood of natural contaminations and outbreaks is increasing as well. Furthermore, if environmental factors are changing, historical anthrax sites might pose additional potential threats. Data on past and current genotypes of B. anthracis in Central Europe, respectively Austria, is very limited. Although anthrax is historically known in Austria, only one clinical isolate from Tyrol has been sequenced so far and genetic information from environmental isolates is literally lacking. This project is expected to increase the knowledge on genotypes of B. anthracis and closely related Bacillus cereus sensu lato (s.l.) strains from various origins, including clinical specimen, specimens from historical collections and soils from anthrax sites. By elucidating present and past genotypes, microevolution and host-adaptation, this project will improve trace back analysis in future outbreak scenarios. The comparison of isolation protocols for different soil types will contribute significantly to CBRN readiness capabilities of partner states. The genetic homogeneity of these bacteria, the resulting challenges regarding characterization and diagnostics of associated infections, requires international collaboration between applied government research institutions and university research.

EP14

Epidemiological study of Influenza viruses and other causative agents of respiratory infection in Khartoum hospitals, Sudan

Mohamed O. Mustafa[1], Khalid Enan[1], R. Surtees [2], A. El Hussein[1], E. Krause[2], N. Hofmann[2], A. Puyskens [2], J. Michel [2], A. Nitsche [2], and I. Elkhidir[1]

1- Central Laboratory, Virology department, Khartoum, Sudan; 2- Robert Koch Institute, Centre for Biological Threats and Special Pathogens, Division Highly Pathogenic Viruses (ZBS1), Seestraße 10, 13353 Berlin, Germany

Background: Respiratory tract infections can be caused by various groups of viruses and bacteria that produce similar signs and symptoms. Etiologic understanding is needed for specific diagnosis; therefore, it relies heavily on laboratory investigation of the causative agent.

Methods: Two hundred and thirty patients experiencing acute respiratory infections between January and March 2015, and August 2015 to March 2016, were enrolled in this study. These were mostly patients admitted to the emergency room (ER) in different Khartoum hospitals. Throat swabs were collected from the enrolled patients and subjected to multiplex real-time RT-PCR assay to detect influenza viruses (subtypes were also determined) as well as other viral and bacterial agents including respiratory syncytial virus (RSV) and human metapneumovirus (hMPV); *Legionella pneumophila*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Sequencing and phylogenetic analysis based on the matrix gene was performed on some of the detected influenza viruses.

Results: Out of 230 patients, 33 were positive for influenza virus infection comprising 22, 10 and 1 patient(s) with influenza types A, B and A and B co-infection, respectively. However no influenza type C was detected. Influenza viruses were detected in 12, 5, 3, 1 and 1 patient(s) in the age groups <2, 2-5, 5-15, 15-49 and >49 years old, respectively.

Out of 230 patients, 46 and 39 were found positive for a different viral pathogen including 38 with RSV and 8 with hMPV infections. On the other hand, bacterial causes of atypical pneumonia including *Legionella, Mycoplasma and Chlamydia* were detected in 27, 9 and 3 patients respectively.

The phylogenetic analysis of the matrix gene sequences of the influenza A virus (H1N1) pdm09 subtype showed these Sudan strains belonged to the some clade and are related to strains from several countries (USA, Ghana, Mexico, India, Thailand, and China). Seasonal influenza A H3 subtypes, have close similarities to strains from Singapore, the USA, South Africa and Australia.

Conclusions: Influenza viruses and other viral and bacterial causes of respiratory infections were detected in a substantial number of acute respiratory infections in Sudan. Determining the causative agent of seasonal respiratory illnesses is important not only for deciding the most effective treatment course, but also to detect potentially novel causes

of acute respiratory illnesses.

EP15

The use of VNTR analysis for assessment of genetic diversity of *Bacillus anthracis* vaccine strains in Ukraine

S. Nychyk, N. Hudz, O. Tarasov, and S. Tereschenko
Institute of Veterinary Medicine NAAS, Kyiv, Ukraine

Introduction: In Ukraine, compulsory vaccinations have reduced anthrax cases in livestock. However, there is still a significant risk of new outbreaks of the disease due to the large number of old cattle burial sites (over 13,500). Data on the genetic variability of Ukrainian strains of *B. anthracis* could help on improving the national vaccination program in Ukraine. Variable number of tandem repeats (VNTRs) is a widely used method for assessing genetic polymorphisms within bacterial species, including *B. anthracis.* Here, we employed VNTR to determine if we could distinguish *Bacillus* strains circulating in Ukraine.

Methods: We used the strains of bacteria: *Bacillus anthracis* K79Z, D-05, UA-07, UA-M, SB-072, Tsenkovskyi-2, Sterne 34F2, as well as *B. cereus var. anthracis*, *Bacillus subtilis* 7241, *Bacillus cereus* 2527, *Bacillus thuringiensis.* DNA from each isolate was obtained using a commercial DNA preparation kit (Thermofisher). VNTR analysis was carried using previously described primers and protocol (Keim et al., 2000) for eight VNTR loci, including pXO1 and pXO2 plasmid sequences.

Results: The primers allowed detection of diverse targets (chromosomal and plasmid) of tested strains of *Bacillus* genus. The test was based on seven marker loci (chromosomal vrrA, vrrB1, vrrB2, vrrC1, vrrC2, plasmid pXO1-aat, and pXO2-at) allowing for differentiation of *Bacillus* species. VNTR markers had high genetic stability, making test highly reproducible. *B. cereus* and *B. thuringiensis* shared 3 markers (vrrA, vrrB1, vrrB2) with *B. anthracis* amplified by PCR, but amplicon sizes were bigger than those from *B. anthracis* strains (rrA-387-462 bp; vrrB1- 211-382 bp; vrrB2-197-492bp).

All five tested chromosomal vrr markers were identified and had almost equal size (vrrA 277-298 bp (â^† = 20); vrrB1 184-192 bp (â^† = 8); vrrB2 153-160bp (â^† = 8); vrrC1 400-468bp (â^† = 12); vrrC2 500-524 bp (â^† = 9) in all *B. anthracis* strains and two plasmid markers were identified only for *Tsenkovskyi-2, Sterne 34F2* strains (pXO1 103-139bp (â^† = 15); pXO2 130-175bp (â^† = 21).

Conclusion: VNTR allows for differentiation of *Bacillus* strains circulating in Ukraine. This method will help to understand genetic diversity of *B. anthracis* strains isolated and can be used for informing the national anthrax vaccination program in Ukraine.

EP16

Catching SARS-CoV-2 by sequence hybridization: a comparative analysis

Alexandra Rehn, Peter Braun, Mandy Knüpfer, Roman Wölfel, Markus H. Antwerpen, and Mathias C. Walter
Bundeswehr Institute of Microbiology, Munich, Germany

Controlling and monitoring the still ongoing SARS-CoV-2 pandemic regarding geographical distribution, evolution and emergence of new mutations of the SARS-CoV-2 virus is only possible due to continuous next-generation sequencing (NGS) and sharing sequence data worldwide. Efficient sequencing strategies enable the retrieval of increasing numbers of high-quality, full-length genomes and are hence indispensable. Two opposed enrichment methods, tiling multiplex PCR and sequence hybridization by bait capture, have been established for SARS-CoV-2 sequencing and are both frequently used, dependent on the quality of the patient sample and the question at hand. Here, we focused on the evaluation of the sequence hybridization method by studying five commercially available sequence capture bait panels in regard to sensitivity and specificity. We discovered the SARS-CoV-2 specific panel of Twist Bioscience to be the most efficient panel, followed by two respiratory panels from Twist Bioscience and Illumina, respectively. Our results provide on the one hand a decision basis and furthermore a guide for using the full capacity of the flowcell for the sequencing community worldwide and on the other hand potential suggestions for improvements for the manufacturers.

EP17

Recording Sporulation Events in *Bacillus subtilis*

Corinna I. Schwarz
Max-Planck-Institute for Evolutionary Biology, Evolutionary Cell Biology, Plön, Germany

Endospore-forming bacteria can alternate between a vegetative reproductive cycle in which a single cell produces two metabolically active progeny cells and a sporulation-germination cycle that includes the formation of a resilient spore to ensure long-term

survival during extended periods of unfavorable environmental conditions. In the presence of the appropriate signals, spores can germinate to regenerate vegetative cells. Vegetative cells and spores are readily distinguished, but it is challenging to distinguish between vegetative cells that originate through cell division and vegetative cells that arise through spore germination. This has complicated the study of how the sporulation-germination cycle is integrated into the overall life cycle of spore-forming bacteria. To address this limitation, I have engineered a strain of *Bacillus subtilis* in which cells that sporulate and then germinate during an experimental regime become fluorescent. The "memory cassette" consists of a *gfp* gene with a constitutive promoter that is disrupted by an antibiotic-resistance gene flanked by a pair of *loxP* sites. Cre recombinase, which mediates a site-specific recombination between the two *loxP* sites, is controlled by a sporulation-specific promoter. This design ensures an excision of the antibiotic resistance gene, and by this the constitutive expression of *gfp*, only in cells that sporulate. Thus, cells that have gone through a sporulation-germination cycle will be green, while cells that did not sporulate will not be green. The fraction of vegetative cells in a culture that arose from one or more rounds of sporulation can be detected by microscopy, flow cytometry or plating. This strain will enable us to study the sporulation-germination cycle very precisely and to investigate the triggers for sporulation in complex environments.

EP18

Screening of old potentially-contaminated anthrax animal burial sites in Eastern and Southern Ukraine

Borys T. Stegniy[1], Oleksandr V. Biloivan[2], Mykhailo V. Gladiy[3], Vasyl V. Vlizlo[3], Anton P. Gerilovych[5], Vasyl L. Arefiev[2], Claudia Popp[6], Julia Schwarz[6], and Gregor Grass[6]
1- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Director, Kharkiv, Ukraine; 2- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Genetics of Microorganisms, Kharkiv, Ukraine; 3- National Academy of Agrarian Sciences of Ukraine, Kyiv , Ukraine; 5- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Veterinary Epizootology, Diagnostics and Quality Control of Genetic Resources, Kharkiv, Ukraine; 6- Bundeswehr Institute of Microbiology, Munich, Germany

Introduction: Anthrax is a dangerous zoonotic disease caused by the spore-forming bacterium *Bacillus anthracis*. Anthrax spores can remain viable for decades in the soil. For the last century, anthrax outbreaks have been registered among livestock animals throughout the entire territory of Ukraine. Today, old animal burial sites of anthrax infected animal carcasses pose a particular risk as natural reservoir of the anthrax pathogen and require constant monitoring by local state laboratory institutions.

Objective: The aim of this study was to screen soil samples of 17 anthrax burial sites in Eastern and Southern Ukraine for the presence of *B. anthracis.*

Methods: Soil samples were collected from anthrax burial sites located in Kharkiv, Sumy and Mykolaiv regions (diseased animals dated from 1946 to 2003). Enrichment and cultivation of *B. anthracis* from collected soil samples was performed with the established GABRI method. Single colonies exhibiting no hemolysis were inactivated with 0.5% peracetic acid/ethanol solution, DNA was extracted and analyzed by qPCR for the presence of chromosomal marker *dhp61*, as well as the markers *pagA* and *capC* located on virulence plasmids pXO1 and pXO2, respectively.

Results: Eleven field trips were conducted from July 2016 to October 2018 in which 369 soil samples from 17 burial sites in Kharkiv, Sumy and Mykolaiv oblasts were collected from different depths of presumed anthrax burial sites. In most cases (12 out of 17 cases), the current status of these sites was deteriorated and not properly accounted for. A single viable *B. anthracis* isolate was obtained from 50 cm depth at the grave site near Koviagy village, Valky district, Kharkiv region (49.92373°N, 35.48951°E). The contamination level of soil at the isolation site reached about total bacterial 10 extsuperscript4 CFU per g as determined by plate counting. qPCR analysis of this isolate identified both the *dhp61 B. anthracis* chromosomal and the pXO1 virulence plasmid marker *pagA*. However, pXO2 plasmid marker *capC*, required for capsule-formation was absent.

Conclusions: Isolation of *B. anthracis* in one case in the Valky district shows that old anthrax burial sites (13.500 exist in Ukraine) may still pose a risk as potential source of the anthrax pathogen and therefore require continued attention and surveillance, for which a surveillance plan will be developed.

FP1

Respiratory pattern parameters in people with borderline personality disorder

Ina Tabirta
USMF, Fiziologie, Chisinau, MDA

Introduction: Respiratory pattern study could pro-

vide the doctors, especially primary level ones, with alternatives in pharmacological treatment, correcting the psychophysiological mechanisms of developed systemic dysfunctions, taking into account 6% BPD sampling at medical assistance level and up to 20% of considerable illnesses caused to the patients in specialized psychiatric centers,.

Materials and methods: The BPD -5 questionnaire on respiration parameters using VisuResp plethysmograph (France), based on electromagnetic inductance and capnographCapnoStream 20 (Sweden) was realized.

Results: It was established, according to the researches, that comparing the mean values â€‹â€‹we can see the respiratory pattern parameters statistical differences in BPD group at rest compared to the control group: the current volume - 21% lower in BPD group, inspiration duration - 10,3% lower in BPD group, respiratory cycle length - 12.1% lower, but the respiratory frequency is 11.5% higher in BPD group. During hyperventilation that occurs with 20 respirations frequency per minute and in the post-hyperventilation condition there is no big difference in respiratory pattern parameters between BPD group and control group. Some differences between respiratory pattern in spontaneous breathing at rest and BPD group post-hyperventilation are observed. The statistical differences are the following ones: current volume is 21.5% higher in post-hyperventilation, inspiration duration is 7.2% less in post-hyperventilation, expiration length is 52.1% higher in post-hyperventilation, total respiratory cycle duration is 33.9% lower in post-hyperventilation, respiratory frequency is 15.5% lower in post-hyperventilation, the mean inspiring flow - 13.1% higher in post-hyperventilation, CO2 concentration is 17.3% lower in post-hyperventilation. As a consequence, can be mentioned that hyperventilation has a strong impact on the respiratory pattern in the people with BPD compared to the control group.

Conclusion: People with BPD small volumes breathing have no differences in respiratory flow, more frequently than the control group. The hyperventilation test reveals some changes in the respiratory patterns of healthy people compared to people with borderline personality disorder probably these changes are caused by the changes in cortical and subcortical structures that are responsible for voluntary and involuntary breath control.

GP1
SARS-CoV-2 pandemic from the Veterinary Perspective

Anja Petrov, U Schotte, K Marquart, and L Johanson

1- Zentrales Institut des Sanitätsdienstes der Bundeswehr, Abteilung A, Kiel, Germany

Coronaviruses of the genus *Betacoronavirus* are the cause of severe infectious diseases in humans in the 21st century culminating in the current SARS-CoV-2 pandemic and are long-known in veterinary medicine. The global spread of SARS-CoV-2, its zoonotic potential, the still unresolved question of an intermediate host as well as the introduction of a mandatory reporting scheme in pets required a SARS-CoV-2 diagnostic also in animals.

Up to now, many efforts were undertaken to establish a reliable diagnostic by real-time RT-PCR and to investigate the susceptibility of different animal models while preanalytic aspects have been disregarded so far. However, in contrast to human medicine the enormous range of animal specific features might affect the validity of diagnostic methods. In order to reveal species-specific influences on the sampling procedure with subsequent effects on SARS-CoV-2 real-time RT-PCR dogs and sheep were analyzed for the following parameters at the Central Institute of the Bundeswehr Medical Service Kiel, Kronshagen, Germany: different oral swabs types, sample storage conditions, and a pretreatment of samples.

The results indicate the existence of species-specific differences (among each other as well as in comparison to human samples) that influence the subsequent detection of SARS-CoV-2 genome by real-time RT-PCR and might even cause qualitative losses. In consequence, knowledge of species-specific peculiarities is of great importance for an accurate diagnostic as basic prerequisite for the identification of SARS-CoV-2 reservoirs and thus for the protection of human health.

Furthermore, against the background that in contrast to humans *Betacoronaviruses* have been successfully established in animals for several decades, specificity controls should also be taken into account in veterinary SARS-CoV-2 diagnostics. Therefore, a pan-*Betacoronavirus* RT-PCR was established in order to detect the *Betacoronavirus* distribution within the animal population and to enable a differentiation of SARS-CoV-2 from other *Betacoronaviruses*. These investigations are ongoing and already comprised several samples of service animals of the Federal Armed Forces.

An extension of that study to wild animal samples that are collected during the national annual monitoring program as well as to further wild animal species that could serve as potential virus reservoir abroad is intended.

HP1

Increased Seroprevalence of *Campylobacter jejuni*, but not HEV in healthcare workers of gastroenterology

Werner Dammermann[1], Andrea von Menges[2], Katrin Singethan[1], Sven Pischke[3], Oliver Ritter[1], Stefan Lüth[1], and Sebastian Ullrich[1]
1- University Hospital Brandenburg, Brandenburg Medical School, Center for Internal Medicine II, Brandenburg an der Havel, Germany; 2- University Medical Center Hamburg-Eppendorf, Department of Anatomy and Experimental Morphology, Hamburg, Germany; 3- University Medical Center Hamburg-Eppendorf, 1. Department of Medicine, Hamburg, Germany

Background: The exposure of healthcare workers (HCW) to fecal-orally transmitted pathogens like hepatitis E Virus (HEV), *Campylobacter jejuni* or *Helicobacter pylori* is still not known. The potential risk for employees or patients to acquire these infections through asymptomatic infected healthcare personnel has not been studied so far. Physicians and nurses in the field of gastroenterology working in endoscopic work spaces were recruited. Employees from the field of cardiology were recruited as well and served as a control group presumed to possess a lower exposure. The cytomegalovirus (CMV) seroprevalence was analyzed as a control pathogen without fecal-oral route of transmission. This study provides an objective view onto the potential exposure risk for healthcare workers (HCW) and patients in endoscopic work spaces. We hypothesize that HCW in the field of gastroenterology show a higher seroprevalence for fecal-oral pathogens like HEV, *C. jejuni* and *H. pylori* compared to HCW in the field of cardiology.

Objective: Primary objective was the assessment of antibody titers against HEV, *C.jejuni* and *H.pylori* in serum of HCW from the fields of gastroenterology as well as cardiology. As a secondary objective we analyzed the seroprevalence against CMV.

Methods: 65 HCW were recruited from the field of gastroenterology (n=42) and cardiology (n=23) in three medical centers in the German federal states of Schleswig-Holstein, Hamburg as well as Brandenburg. Antibody titers were determined via ELISA in serum.

Results: HCW from the field of gastroenterology showed a significantly higher *C.jejuni* seroprevalence for IgG (19.1 %) compared to HCW from the field of cardiology (8.7 %). IgA titers against *C.jejuni* were negligible. HEV seroprevalence for IgG did not differ significantly between HCW from the field of gastroenterology (7.1 %) and cardiology (8.7 %), respectively. IgA and IgM titers against HEV were also negligible. All other antibody titers against CMV and *H.pylori* were without pathological findings and showed no significant difference.

Conclusions: Only *the C.jejuni* seroprevalence was significantly increased in HCW from the field of gastroenterology. HEV seroprevalence showed no differences. The results for CMV and *H.pylori* were without pathological findings. There is no job-related exposure risk regarding HEV, but for *C.jejuni* the protective measures might need to be improved.

HP2

Distinct differences in the growth patterns of SARS CoV-2 IMBmuc1 and IMB CB B1.1.7 Kent variant in various cell lines give possible explanation for differences in transmissibility

Friederike Hucke, Frank Schwarze, Natascha Bayer, Roman Woelfel, and Joachim Bugert
Bundeswehr Institut of Microbiology, Munich, Germany

Both, the first sample of Wuhan Type SARS CoV-2 (IMB muc 1-4; 'muc1') and the first UK Kent 'Variant of Concern' (VoC; IMB CB B1.1 .7; "CB") were isolated at the Bundeswehr Institute of Microbiology, shortly after their first appearance in Germany in January 2020 (muc1) and December 2020 (CB).

The growth patterns of these two virus variants (passaged twice in VeroE6 cells after their isolation) were investigated under identical conditions in different cell lines and virus yield in the supernatant was measured and compared (n=1). While both SARS-CoV-2 variants showed similar virus production with cytolysis (cytopathic effect = CPE) in VeroE6 cells, human A549-ACE2 cells can be infected with muc1 but do not yield virus into the supernatant. The Kent variant CB does yield less virus into the supernatant of A549-ACE2 cells than in VeroE6 cells but is considerably more productive in this lung cell line than muc1. This observation was confirmed in the higher differentiated human lung cell line Calu3. Muc1 infects this cell line without yielding virus into the supernatant while CB virus production in Calu3 reaches a level comparable with the isolation cell line VeroE6. Both SARS-CoV-2 variants show no CPE in either lung cell line. Thus the two lung cell lines (A549-ACE2 and Calu3) are not suitable for CPE based endpoint titration assays such as the virus neutralisation test (VNT) established in VeroE6 cells. Both virus variants infected human colorectal epithelial cells (Caco-2) with virus production into the supernatant, although CB yielded notably more virus particles than muc1.

Keeping in mind the limited predictive value of in vitro experiments in immortalised cell lines and possible changes in n=3, we believe our results provide an explanation for the higher transmission of SARS-CoV-2 Kent variant CB in human infections versus muc1: Virus production into the cell surroundings of human epithelial lung cells is considerably higher with CB than with muc1. In vivo, the higher virus yield would correlate with a higher virus production in the upper airways and thus with the increased transmission via droplets and aerosols. With the same reservation, the possible secretion of infectious SARS-CoV2 CB, and possibly more recent variants with higher transmissibility in the faeces of COVID patients must be considered and formally investigated as this might pose a way of transmission which seemed not important in the original SARS-CoV-1 variant muc1.

HP3

Human IL-6- or IL-3/GM-CSF-transgenic NOG mice transplanted with human hematopoietic stem cells as a promising candidate model of lethal *Staphylococcus aureus* enterotoxin B shock

Masahiro Nakashima[1], Kimiya Sato[2], Hiroyuki Nakashima[1], Kazuki Koiwai[1], Nariyoshi Shinomiya[3], and Manabu Kinoshita[1]
1- National Defense Medical College, Immunology and Microbiology, Tokorozawa, Japan; 2- National Defense Medical College, Basic Pathology, Tokorozawa, Japan; 3- National Defense Medical College, President, Tokorozawa, Japan

Staphylococcus aureus enterotoxin B (SEB) is a virulent superantigen. Inhalation of this toxin even at microgram levels can be lethal for humans. Since SEB shows a low affinity for rodent MHC class II, with little effect on mice, there are no good *in vivo* models for investigating effective countermeasures for lethal SEB shock. We explored the utility of human IL-6 or IL-3/GM-CSF transgenic NOD/SCID/γ_c^{null} (NOG) mice transplanted with human hematopoietic stem cells, showing a higher engraftment ratio of human leukocytes and more human-like reactions to SEB than previous models. After intratracheal exposure to SEB, these humanized mice had markedly elevated levels of human cytokines, including IL-2, but not murine cytokines. Notably, no humanized IL-6 transgenic NOG mice survived after SEB exposure. These findings demonstrated the validity of humanized mice for the *in vivo* analysis of lethal SEB shock, thus leading to the further development of medical countermeasures against such intoxication.

IP1

Self-sampling versus health care professional-guided swab collection for SARS-CoV-2 testing

Silvia Würstle[1], Christoph D. Spinner[1], Florian Voit[1], Dieter Hoffmann[2], Svenja Hering[2], Simon Weidlich[1], Jochen Schneider[1], Alexander Zink[3], Matthias Treiber[1], Roman Iakoubov[1], Roland M. Schmid[1], Ulrike Protzer[2], and Johanna Erber[1]
1- School of Medicine, University Hospital Rechts der Isar, Technical University of Munich, Department of Internal Medicine II, Munich, Germany; 2- Technical University of Munich, Institute of Virology, Munich, Germany; 3- School of Medicine, University Hospital Rechts der Isar, Technical University of Munich, Department of Dermatology and Allergology, Munich, Germany

Purpose: To evaluate the diagnostic reliability and practicability of self-collected oropharyngeal swab samples for the detection of SARS-CoV-2 infection as self-sampling could enable broader testing availability and reduce both personal protective equipment and potential exposure.

Methods: Hospitalized SARS-CoV-2-infected patients were asked to collect two oropharyngeal swabs (SC-OPS1/2), and an additional oropharyngeal swab was collected by a health care professional (HCP-OPS). SARS-CoV-2 PCR testing for samples from 58 participants was performed, with a 48-h delay in half of the self-collected samples (SC-OPS2). The sensitivity, probability of concordance, and interrater reliability were calculated. Univariate and multivariate analyses were performed to assess predictive factors. Practicability was evaluated through a questionnaire.

Results: The test sensitivity for HCP-OPS, SC-OPS1, and SC-OPS2 was 88%, 78%, and 77%, respectively. Combining both SC-OPS results increased the estimated sensitivity to 88%. The concordance probability between HCP-OPS and SC-OPS1 was 77.6% and 82.5% between SC-OPS1 and SC-OPS2, respectively. Of the participants, 69% affirmed performing future self-sampling at home, and 34% preferred self-sampling over HCP-guided testing. Participants with both positive HCP-OPS1 and SC-OPS1 indicating no challenges during self-sampling had more differences in viral load levels between HCP-OPS1 and SC-OPS1 than those who indicated challenges. Increasing disease duration and the presence of anti-SARS-CoV-2-IgG correlated with negative test results in self-collected samples of previously confirmed SARS-CoV-2 positive individuals.

Conclusion: Oropharyngeal self-sampling is an appli-

cable testing approach for SARS-CoV-2 diagnostics. Self-sampling tends to be more effective in early versus late infection and symptom onset, and the collection of two distinct samples is recommended to maintain high test sensitivity.

IP2

Twice SARS-CoV-2 positive PCR-test Surveillance system: can it be useful for detection of COVID-19 reinfection, Armenia, 2021

Gennady Palozyan[1], Romella Abovyan[1], and Artavazd Vanyan[2]
1- NCDC, MoH of Armenia, Department of Epidemiology of Infectious and Non-Communicable Diseases, Yerevan, Armenia; 2- NCDC, MoH of Armenia, Yerevan, Armenia

Introduction: The COVID-19 pandemic is one of the 21st century"s most severe challenges. At present, there are more than 140 million people infected and 3 million people have died. Confirmation of SARS-CoV-2 reinfection requires complex laboratory and financial support, especially in middle- and low-income countries. Alternative methods are needed to make detection of suspected cases of reinfection more manageable. The aim of this study was to develop an operational case definition for evaluation of twice positive SARS-CoV-2 PCR-test surveillance system to detect suspected cases of reinfection.

Methods: From 03/2020-03/2021, more than 600 cases were identified in the national electronic healthcare system ARMED of patients testing positive twice by PCR with at least a 90-day interval between positive tests. We conducted a random survey of 301 persons using a standardized questionnaire. Out of 301 selected people, 83 (27%) persons refused to answer.

Results: Out of 218 respondents, 93 (43%) were male and 125 (57%) were female. The mean age was 46.4 $\pm$ 0.7 years, and 18 persons had a known comorbidity (diabetes mellitus, cancer, lupus etc.). The average period between first and last positive PCR was 182.8 $\pm$ 5.9 days. After recovery from the 1 extsuperscriptst episode, there were at least 60 asymptomatic days before a 2 extsuperscriptnd episode in all cases with 64 persons having a confirmed negative by PCR after 1 extsuperscriptst episode. The 1 extsuperscriptst episode had mild/moderate/severe course in 76/39/2 cases, with 101 (46%) individuals showing no symptoms. The 2 extsuperscriptnd episode had mild/moderate/severe course in 86/79/10 cases, with 43 (20%) individuals presenting no symptoms. In 17 (8%) cases both episodes were asymptomatic. In 91 (42%) cases both episodes were symptomatic, including 9 (4%) patients, who had two episodes of pneumonia.

Conclusion: Based on the results of the study, the following suspected case definition was developed: at least a 90-day interval between first and last positive PCR AND at least 60 days of asymptomatic period before 2 extsuperscriptnd episode AND [1 extsuperscriptst symptomatic episode OR presence of anti-COVID-19 IgG after 1 extsuperscriptst episode] AND 2 extsuperscriptnd symptomatic episode. This case definition could be used to identify suspected cases to be further sequenced to confirm SARS-CoV-2 reinfection.

IP3

Comparing a telemedicine-guided self-collection approach to regular PCR-based SARS-CoV-2 testing in a large European industrial manufacturing company

Silvia Würstle[1], Johanna Erber[1], Michael Hanselmann[1], Dieter Hoffmann[2], Stanislav Werfel[3], Svenja Hering[2], Simon Weidlich[1], Jochen Schneider[1], Ralf Franke[4], Michael Maier[4], Andreas Henkel[5], Roland M. Schmid[1], Ulrike Protzer[2], Michael Laxy[6], and Christoph D. Spinner[1]
1- School of Medicine, University Hospital Rechts der Isar, Technical University of Munich, Department of Internal Medicine II, Munich, Germany; 2- Technical University of Munich, Institute of Virology, Munich, Germany; 3- School of Medicine, University Hospital Rechts der Isar, Technical University of Munich, Department of Nephrology, Munich, Germany; 4- Siemens AG, Frauenauracherstrasse 80, Erlangen, Germany; 5- School of Medicine, University Hospital Rechts der Isar, Technical University of Munich, Department of Information Technology, Munich, Germany; 6- Technical University of Munich, Public Health and Prevention, Munich, Germany

Background: Large-scale SARS-CoV-2 testing using PCR is expensive. Self-collection approaches represent an alternative but require evaluation regarding feasibility, expenses, and infection prevention benefits.

Aim: To compare an innovative PCR-based self-collection approach to regular SARS-CoV-2 testing in a large European industrial manufacturing site.

Methods: The feasibility of a telemedical PCR-based self-collection approach was assessed in 150 employees (intervention group) and compared to regular SARS-CoV-2 testing at a test centre (n=143, control group). Acceptance, ergonomics, and efficacy were evaluated using a software application. A sim-

ulation model was applied to evaluate test strategy effectiveness.

Results: The test results were successfully transmitted and interpreted without uncertainty by 76% of the intervention and 76.9% of the control group (p=0.89). Ratings of acceptability, ergonomics and efficacy of the intervention group were non-inferior compared to the control group (acceptability: 71.6 vs. 37.6%; ergonomics: 88.1 vs. 74.5%; efficacy: 88.4 vs. 77.5%). The self-collection approach was estimated to take less time (23 vs. 38 minutes, p>0.001). A simulation model indicated that both testing approaches reduce the infection risk and that the self-collection approach tends to be slightly less effective due to lower sensitivity. An interactive R shiny app was built to enable customized simulations (available at: https://mh-php-tum.shinyapps.io/Covid_19_Test_Strategy_Simulation/.

Conclusion: ThePCR-based self-collection approach for SARS-CoV-2 diagnostic is technically feasible and rated well regarding acceptance, ergonomics, and efficacy. Simulation models are helpful to evaluate test effectiveness but should be adapted for individual companies" context specificity.

IP4

Detection of *Legionella* spp. and *Legionella pneumophila* in water and air samples using culture and viability-qPCR

Mihai Zamfir, Sandra M. Walser-Reichenbach, Stefanie Heinze, and Caroline E.W. Herr
Bavarian Health and Food Safety Authority, Occupational and Environmental Health, Epidemiology, Munich, Germany

Legionella are naturally occurring water bacteria that enter technical water systems from the environment. Inhalation of Legionella-containing bioaerosols can lead to Pontiac fever or Legionnaires' disease in humans. From 1998 to 2020, 33 documented *Legionella* outbreaks associated with evaporative cooling systems, cooling towers, or wet scrubbers were identified internationally. To minimize the risk of such outbreaks, the German government enacted the regulation on evaporative cooling systems, cooling towers and wet separators (42. BImSchV), which took effect in 2017. The identification of a possible source is typically done by the culture method (ISO 11731) which requires 10 days and is often hindered by the background microbial flora growth.

In an effort to establish a catalogue of measures, which contributes to contain the threat to civil security as quickly as possible, we have evaluated molecular and immunological approaches to detect, quantify and characterize *Legionella* in water and air environmental samples.

Water and air samples were screened for *Legionella* spp. and *L. pneumophila*. The samples were analyzed in parallel with standard culture methods and with viability-qPCR using propidium monoazide (PMA). Through treatment with PMA we were able to differentiate between cells with an intact membrane (viable) and those with a damaged membrane (non-viable). The concentration of PMA was adjusted based on the bacterial and organic load of the samples.

In total, 107 water samples and 28 air samples were collected from various sample matrices. Using qPCR, *Legionella* spp. was found in 115 samples. *L. pneumophila* was found in seven cooling towers, three wastewater treatment plants, and two industrial-processes water samples in concentrations between 100-25000 GU/100 mL and between 3-550 GU/ m extsuperscript3 in three air samples. Using the culture method, only seven samples were positive for *L. pneumophila*.

qPCR detection of *Legionella* spp. and *L. pneumophila* shows higher sensitivity than culture based methods and may be especially useful in detecting *Legionella* containing aerosols. The methods described have been mentioned as part of the VDI (Verein Deutscher Ingenieure) guideline: "Catalogue of measures in case of suspicion of emission-related legionellosis outbreaks - Identification and investigation of aerosol-emitting environmental sources in the context of legionellosis outbreaks."

IP5

Detection of *Legionella* in water and air samples from car washes using culture method and qPCR

Johannes Redwitz, Mihai Zamfir, Sandra M. Walser-reichenbach, Bernhard Brenner, Stefanie Heinze, and Caroline E.W. Herr
Bavarian Health and Food Safety Authority, Occupational and Environmental Health, Epidemiology, Munich, Germany

Legionella occur in water bodies from which they can enter artificial water-bearing systems and multiply under the right circumstances. There is a risk of infection when these systems subsequently aerosolise the water, which makes an entrance of *Legionella* into the airways and therefore illness possible.

In car washes a large part of the water used is aerosolised. In addition, the use of recycled water that is not commonly treated with biocides and

sometimes heated, can promote an increase in the *Legionella* concentration. In customers of different car wash installations, cases of legionellosis have been documented and could be associated with the use of the car wash (Baldovin et al., 2018; Euser et al., 2013). Employees in particular are repeatedly exposed for longer. Therefore, the aim of the current pilot study is to determine the risk of infection for employees that work at or near car washing facilities.

Eight car washes were sampled for these investigations. Water samples were collected from freshwater, the tank with recycled water, and the connected pressure washer. Furthermore, air samples were collected downwind, at the workplace, and inside the car wash using the Coriolis μ sampler.

For analysis of the samples, established culture-based methods following a protocol adapted from ISO 11731 were used. For comparison, molecular methods such as qPCR and viability-qPCR for live/dead differentiation were applied. With the culture method *Legionella* spp. were detected in the water samples of only one car wash but using qPCR *Legionella* spp. were detected in a large proportion of the water samples. Moreover, viability-qPCR showed that in these samples viable cells were present in high concentrations at the time of sampling. The air samples did not test positive for *Legionella* with the culture method. In contrast, when using qPCR, more than half of the car wash samples showed quantifiable concentrations of viable *Legionella* spp. The samples collected at workplace of these car washes often tested positive for viable *Legionella* spp. but mostly at concentrations below the limit of quantification.

Medically most relevant species, *Legionella pneumophila* was not detected during these investigations. However, since the conditions for the occurrence of *L. pneumophila* and *Legionella* spp. are similar, an occurrence of *L. pneumophila* in car washes is possible and additional car washes are going to be investigated as part of the project to gain more insight.

IP6

Rapid Outbreak Response and Future Pandemic Preparedness by Molecular Workarea (MWA) PCR Diagnostics

Bodo Eickhoff
Roche Diagnostics Deutschland GmbH, Molecular Diagnostics, Mannheim, Germany

Outbreaks of new emerging viruses, like SARS-CoV2, require fast and robust PCR assay development with broadly available open Diagnostic platforms for rapid containment. In case of developing into a global pandemic, fast production scale-up and standardised automated mass testing are needed across the globe. Fully integrated, sample-to-result-automated walk-away Molecular Workarea solutions will help coping with the massive numbers of samples and necessary routine testing, especially in scenarios with limited staff availability. New high throughput stationary system concepts will be presented.

IP7

Protective Antigen (PA) specific ELISA with Bacillus expressed recombinant antigen

Peter Braun[1], Eva-maria Kalbhenn[1], Sabine Zange[2], Gregor Grass[1], and Heiner Von buttlar[1]
1- Bundeswehr Institute of Microbiology, Bacteria and Toxins, Munich, Germany; 2- Bundeswehr Institute of Microbiology, Central Diagnostic Unit, Munich, Germany

Bacillus anthracis with its spore forming capacity is one of the biological agents with the highest potential to be misused. It facilitates its pathogenic effects by the production of two toxins, the edema toxin and the lethal toxin. Both of them are composed of two proteins, the lethal factor (LF) or the edema factor (EF) that are shuttled into the host cell by the pore forming protective antigen (PA). Therefore, vaccines used to prevent disease mainly rely on the induction of PA-specific antibodies to neutralise the *B. anthracis* toxins. The detection of PA specific antibodies is used for serological diagnostics, especially to verify vaccination success. Here we present the production of recombinant antigen for ELISA using a *Bacillus subtilis* expression system. Since PA is also the target gene of PCR for the detection of *B. anthracis*, we modified the coding sequence to prevent diagnostic primers to bind to the plasmids employed for protein expression. The protein was fused to a Strep-tag sequence for the preparation of highly purified antigen. This material was used to set up an ELISA to detect PA specific antibodies in vaccinees' sera. Thus, we demonstrated the usefulness of a gram-positive expression system for the production of recombinant antigens for serodiagnostic applications.

IP8

Detection of respiratory pathogens in adult patients with suspected COVID-19 in Kuala Lumpur, Malaysia

Yoong M. Chong[1], Yoke F. Chan[1], Mohamad F.H. Jamaluddin[2], Mohd S. Hasan[2], Yong K. Pang[3],

Sasheela Ponnampalavanar[3], Sharifah F. Syed Omar[3], and I C. Sam[1]
1- University Malaya, Medical Microbiology, Kuala Lumpur, Malaysia; 2- University Malaya, Anesthesiology, Kuala Lumpur, Malaysia; 3- University Malaya, Medicine, Kuala Lumpur, Malaysia

During the COVID-19 pandemic, circulation of other common respiratory viral pathogens can potentially result in co-infections; however, reported rates of co-infections for SARS-CoV-2 vary and limited information is available in developing countries. In this study, we investigated the incidence of respiratory pathogens in adults admitted with suspected COVID-19 in Kuala Lumpur, Malaysia.

From February to May 2020, 198 adult patients with acute respiratory infection (ARI) and suspected COVID-19 admitted to University Malaya Medical Center in Kuala Lumpur, Malaysia, were included. Respiratory samples were tested using SARS-CoV-2 RT-qPCR assay and Luminex NxTAG-RPP assay (20 common respiratory pathogens). MERS-CoV, SARS-CoV and bacterial select agents (*Burkholderia pseudomallei*, *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*) were also tested. Forty-five samples with negative findings were further tested with viral metagenomics to capture the potential respiratory pathogens missed out by these molecular assays.

Of the 198 samples, 74 samples (37.4%) had respiratory pathogens. After SARS-CoV-2 (28.3%; 56/198), rhinovirus/enterovirus (5.6%; 11/198) was the next most detected common respiratory virus, followed by parainfluenza virus (1.5%; 3/198). Three negative samples (6.7%; 3/45) had viruses identified by viral metagenomics, of which two were respiratory related (enterovirus D68 and Saffold virus) and human papillomavirus type 4. About 8.9% (5/56) of SARS-CoV-2 positive patients were co-infected with rhinovirus/enterovirus and none of the cases had positive blood cultures. None of the samples were positive for SARS-CoV, MERS-CoV and bacterial select agents. Most of the COVID-19 patients (91.1%; 51/56) had mild symptoms. Detection of respiratory pathogens (other than SARS-CoV-2) was significantly associated with asthma, chronic lung disease, severity of illness, shortness of breath and positive blood cultures.

It is important to continue surveillance for common respiratory pathogens, which still account for 9.1% (18/198) of hospitalization cases with ARI. However, co-infections with SARS-CoV-2 were rarely detected, with no observed mortality. Surveillance for respiratory pathogens using metagenomics analysis can identify unexpected or rare pathogens. Although *B. pseudomallei* is endemic in Malaysia, the presence of select agents appears to be rare in Malaysia. Further studies with larger sample sizes and multiple geographical regions are needed.

IP9
Swabs for sampling biological agents

Christine Eschmann, Sylvia Schirmer, and Stefan Köhne
Wehrwissenschaftliches Institut für Schutztechnologie, Detection WIS220, Munster, Germany

The detection of biological warfare agents is an important capability of CBRN Protection Forces in order to identify potential dangers and to initiate appropriate follow-up measures as early as possible. Sampling as first step of a defined workflow is crucial for the laboratory-based analysis of samples afterwards. A wide range of swabs and wipes of diverse materials are commercially available for various applications. The range of biological agents handled by the CBRN Protection Forces of the Bundeswehr is wide, either. It seems to be obvious that requirements for sampling bacterial material are likely to differ from those for toxins. A variety of transport mediums has been established to preserve different biological agents. However, the material of the sampling device could affect methods of sampling, biological agents and their analysis, too. E.g., a weak or hollow rod of a swap could impair handling, lignin from wooden rods could inhibit bacteria and interfere with PCR, and cotton tips could cause protein background signals in toxin analysis. In addition, swabs and wipes of different materials vary significantly with respect to uptake and release of liquid and biological agents. This is critical to examine, as the recovery of biological agents plays a fundamental role in the detection of CBRN warfare agents. As it comes to sampling methods, liquids for pre-moistening the swab, to transport, solve and store the biological agent could have another impact on the biological agent and their analysis.

This study examines the effects of the sampling device material and the methods of sampling on sample analysis.

IP10
Establishing of the multiplex PCR method for detection of *Clostridium botulinum*

Mari Gavashelidze[1], Roena Sukhiashvili[1], Gvantsa Brachveli[1], Meri Pantsulaia[1], Jana Pollakova[2], Michaela Kreitmeier[2], and Heiner Von buttlar[2]
1- National Center for Disease Control and Public Health (NCDC), Tbilisi, Georgia; 2- Bundeswehr Institute of Microbiology (IMB), Munich, Germany

Clostridium botulinum bacteria is spore-forming, obligate anaerobic, gram-positive rod which produces a neurotoxin (BoNT) and thereby is the etiological agent of botulism. Botulism is an acute neuroparalytic disease caused by the BoNTs, the most potent toxins known. Therefore it might be misused as a biological weapon. BoNTs can be divided in seven antigenic types (A-G), of which human cases are caused primarily by neurotoxin types A, B, E and F. Based on the entry route to the body foodborne, cutaneous, injection or inhalation botulism are known. For the last two years, canned vegetables prepared at home have been the main cause of botulism in Georgia. In 2019, eleven cases were identified and in 2020 nine cases were related to canned vegetables.

National Center for Disease Control and Public Health (NCDC) in Georgia regularly receives clinical samples for the diagnosis of botulism. So far, routine diagnostic is based on isolation, cultivation and confirmation of the BoNTs by mouse bioassay, which is still the gold standard. However, this method is expensive, labour-intensive and slow. Quantitative real-time PCR (qPCR), with its high sensitivity, specificity and short duration, is an essential method for molecular testing in clinical diagnostics. Therefore, our aim is to broaden the routine diagnostics of BoNTs at NCDC by quadruplex real-time PCR assay according to Satterfield et al. 2010. For verification of the qPCR, specifically designed plasmids, containing four BoNTs gene fragments (A, B, E, F) are used as positive controls. The whole process is carried out in the laboratory of the Richard Lugar Public Health Research Center and after successful completion of the procedure, this qPCR will be included to the national laboratory diagnostic algorithm along with serological and bacteriological tests. Overall, this achievement is significant for early and accurate diagnosis, epidemiological surveillance and public health.

This work is part of the German Biosecurity Programme: Establishment of a Western Asian Network for the Improvement of Biosecurity in the Caucasus Region.

IP11

Comparative study of Loop mediated isothermal amplification method for rapid detection of *B. anthracis* in samples with qPCR assay

N. Hudz, S. Nychyk, O. Zaharova, and O. Tarasov
Institute of Veterinary Medicine NAAS, Kyiv, Ukraine

Introduction: Due to the high clinical significance of anthrax in Ukraine, effective methods for rapid detection remains relevant. The high risk for anthrax outbreaks remains due to the large number of old anthrax burial sites and the possible circulation of the pathogen in the wild fauna in Ukraine. The aim of the study was to evaluate the diagnostic efficiency of the loop-mediated isothermal amplification assay (LAMP) for B. *anthracis* compared with commercially available qPCR test kit. LAMP does not require special equipment and the results can be visualized. This makes LAMP an easier and faster field test for pathogen detection.

Methods: Bacterial strains were obtained from IVM NAAS museum: *B. anthracis* K79Z, Tsenkovskyi-2, Sterne 34F2, *B. cereus* 2527, *B. cereus var. anthracis*, *B. subtilis* 7241, *B. thuringiensis*. Bacterial cultures and artificially contaminated soil samples were used for the study. Sensitivity, specificity, and predictive values of LAMP were compared with qPCR. A previously published LAMP oligonucleotide primers for pagA gene (pXO1 plasmid) were compared with the commercial qPCR anthrax detection kit "SibDif" (Amplisense). A commercial DNA preparation kit (Genomic DNA Purification Kit, Thermofisher) was used in our study.

To assess the analytical sensitivity of LAMP and qPCR, serial 10-fold dilutions of bacterial culture were analyzed: from 1x 10^8 to 1x 10^1 CFU/cm^3. For each dilution total DNA was isolated and tested.

Results: Using optimized conditions for LAMP the concentration of 8.0 mM Mg $^{2+}$, Bst polymerase 24 U/20 "l, and 63°C, the reaction time was reduced from 45 to 35 min compared to real-time PCR. Analytical sensitivity of pathogen detection in LAMP was 2.9 pg DNA per reaction and 3.0 pg DNA per soil sample. The diagnostic sensitivity of the LAMP was 96%. Overall agreement among LAMP and qPCR was high (kappa value=1.0). Specificity was similar tests (100%).

Conclusion: The LAMP test allows to detect *B. anthracis* DNA in bacterial culture and soil. The advantages of LAMP over qPCR are the possibility of using it under field conditions. Applying LAMP for anthrax detection in Ukraine will help to evaluate the danger of old anthrax burial sites and improve the risk assessment.

IP12

New possibilities for rapid detection of pathogenic viruses using the surface plasmon resonance

Zinaida S. Klestova[1] and Volodimir P. Maslov[2]
1- State Scientific-Control Institute of Biotechnol-

ogy and Strains of Microorganisms, Biotechnology and antivirus control Dep, Kyiv, Ukraine; 2- V. Ye. Lashkaryov Institute of Semiconductor Physics, National Academy of Sciences of Ukraine, Department of Physical and Technological Fundamentals of Sensory Materials Science., Kyiv, Ukraine

The use of surface plasmon resonance (SPR) in biochemical research and medicine began in 1984. The advantages of the method are high sensitivity, selectivity, and the ability to adjust to different analytes. Using an SPR instrument, we set out to establish a method for detecting various viruses in water and air samples. We hypothesized that coating the sensor with functional groups would increase the sensitivity of the instrument by increasing binding affinity and reducing non-specific interactions. In addition, we restricted mathematical processing of data the initial part of the binding curve to improve the signal. Standard protocols were used for producing, quantifying, and diluting virus. The sensor was 'functionalized' using mix of two solution. First solution is N-ethyl - NêžŒ- (dimethylaminopropyl) carbodiimide hydrochloride 47-77 mg/cm ^{3} in distilled or deionized water. Second solution is N-hydroxysuxinimide 9-14 mg/cm ^{3} in distilled or deionized water. The sensor was kept in a mixture of solutions for 10-20 minutes at a temperature of 25 $\pm$10°C. Samples were analyzed as previously described. Functionalization of the sensor increased enterovirus detection 8-fold. The angular shift of the resonant characteristic for the sensor with a functional coating for serum with antibody diluted 1000 times (Serum 1%) was 9 times greater ($\Delta\theta$ = 155 $\pm$ 3 angular sec.) than for the sensitive element without functional coating, which was $\Delta\theta$ = 17 $\pm$ 3 angular sec. At a higher serum concentration (Serum 10%) the angular shift of the resonant characteristic for the sensitive element with functional coating was 7.8 times greater ($\Delta\theta$ = 1454 $\pm$ 3 angular sec.) than for the sensitive element without functional coating, which was $\Delta\theta$ = 187 $\pm$ 3 corner sec. Coronavirus transmission through drinking water and airborne droplets has been studied. The response of the sensor to the immobilization of the antigen from the pathogenic aerosol (spray) was almost 10 times less (0.05 angular deg. Versus 0.43 angular deg.) than for the initial solution. The response to antigen-antibody binding was almost the same, 0.49 and 0.47 angular degrees, respectively. Application of surface Plasmon resonance device is cheaper and can decrease time of measurement. Application of special coatings on the sensitive element increased sensitivity by 8 times and decreased weak-positive results. Processing of only initial part of the SPR-curve also reduced measurement times.

IP13

Towards faster detection of pathogens in human body fluids: testing platform for affinity ligands for monolithic affinity filtration

Julia Neumair[1], Vivienne Zheng[2], Stephan Heller[2], Silvia Würstle[3], Andreas Obermeier[2], Jochen Schneider[3], Rainer Burgkart[2], Martin Elsner[1], and Michael Seidel[1]

1- Technical University of Munich, Chair of Analytical Chemistry and Water Chemistry, Munich, Germany; 2- Technical University of Munich, Klinikum Rechts der Isar, Experimental Orthopedics, Munich, Germany; 3- Technical University of Munich, Klinikum Rechts der Isar, Klinik und Poliklinik für Innere Medizin II , Munich, Germany

Ascites is the collection of fluid in the peritoneal cavity, which can accumulate in large amounts of several liters and is mostly a consequence of progressive liver cirrhosis. Spontaneous bacterial peritonitis, an infection of ascites without an identifiable and intra-abdominal surgically treatable source, can be caused by Gram-positive and Gram-negative bacteria, as well as fungi. Since their concentration is very low, culture-based methods, still the gold standard for clinical diagnostic, take a long time for a positive result or even give false-negatives results. To avoid an increasing mortality and in respect to antibiotic resistances, a rapid and precise treatment of the infection is necessary. To overcome this problem, a fast and precise detection of the pathogens is required.

One approach is the concentration of the pathogens by monolithic affinity filtration. Here we use self-produced epoxy-based monoliths with a pore size of 22 "m. The body fluid passes the porous filter and the pathogens interact in the inside with present ligands via affinity. By changing the pH, protein and salt content the affinity can be disturbed and elution of the pathogens in a smaller volume is possible. These ligands can be antibiotics, proteins, and possibly even bacteriophages. To identify suitable ligands, we are developing a 2D testing platform. The substances to be examined are therefore immobilized on modified polycarbonate surfaces. Assembled to a microfluidic chip, a flow-through assay is performed on an automated platform. Biotin-labeled pathogens can bind to the immobilized affinity (bio-)ligands and are detected by a horseradish peroxidase-labeled streptavidin through a chemiluminescence reaction. By flushing the flow cell with an eluent and detection of the remaining bound pathogens the desorption can be observed.

First, Polymyxin B, a cationic, cyclic antibiotic was tested with *Escherichia coli* and eluated with a 1:50

dilution of carbonate buffer. Here, binding of *E. coli* as well as a decrease in signal of around 38% after elution was observed, showing a desorption of the cells. With this testing platform different bioligands like Vancomycin, lysozyme, Concanavalin A and possibly bacteriophages can now be tested in body fluids and with different pathogens. After identifying the best ligand and eluent, the whole system will be transferred on the monoliths and tested with real samples to increase the detection effectivity of pathogens in human body fluids.

IP14

Fluorescein copolymers improve counter-immunoelectrophoresis (CIE) diagnostics

Dmytro Ostapiv[1], Natalia Kuz'mina [1], Mariya Kozak[1], Vasyl Vlizlo[2], Igor Kotsiumbas[3], Sergij Varvarenko[4], Volodymyr Samaryk[4], Natalja Nosova[4], Marija Yakoviv[4], and Yurij Kosenko[3]
1- Institute of Animal Biology, Lviv, Ukraine; 2- Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine; 3- State Scientific-Research Control Institute of Veterinary Medicinal Products and Feed Additives, Lviv, Ukraine; 4- L'viv Polytechnic National University, Lviv, Ukraine

The appearance of new strains of pathogens predetermines both the improvement of existing, and the search for new, rapid diagnostic tests for infectious diseases. Counter-immunoelectrophoresis (CIE) is a highly specific, simple and rapid method, which does not require expensive equipment, input materials, or long-term staff training as compared with other rapid diagnostic methods for infectious agents. The sensitivity of CIE can be weak due to the low levels of specific antibodies. Polymers based on pseudo-polyamino acids can bind proteins. Therefore, the purpose of the study was to improve CIE method by strengthening the antigen-antibody interaction with the use of synthesized amphiphilic fluorescein-containing copolymer. Polymeric product based on lipophilic 2-(octadecanoylamino) pentanedioic acid, hydrophilic polyoxyethylene diol with a molecular weight of 1000 or 1500 dipropylene glycol, and fluorescein was synthesized. This copolymer has a complex self-organized structure with a gradient of lipophilic-hydrophilic properties and can solubilize a significant amount of protein. This copolymer binds up to 70% of blood proteins. The synthesized complex is a fluorogenic marker for obtaining images in the visible part of the light spectrum. It was demonstrated that the use of the fluorescein copolymer-antigen complex enhanced the interaction with serum/ plasma antibodies of rabbits immunized against hemorrhagic disease and increased protein content in the area of precipitation. This effect was more pronounced when more hydrophobic copolymer containing 5.87% fluorescein was introduced into the complex with antigen. The antigen-antibody complex content in the fluorescein copolymer complex confirms that fluorescein copolymer addition to the antigen makes the appearance of precipitation zones more intense. The ability of the amphiphilic fluorescein copolymer to enhance the antigen-antibody interaction and the ability to visualize the fluorescent precipitation zone may increase CIE effectiveness for express diagnostic of infectious diseases. In the future, it is planned to test this method for diagnosing of other infectious diseases.

IP15

Effects of Reactive Skin Decontamination Lotion (RSDL®) on Ricin Activity

Vladimir Savransky[1], Marloes Joosen[2], Daan Noort[2], Mulu Gebremedhin[1], Laura Cochrane[3], and John Barry[1]
1- Emergent BioSolutions, Research and Development, Gaithersburg, USA; 2- TNO Defence, CBRN Protection, Rijswijk, Netherlands; 3- Emergent BioSolutions, Medical Affairs, Tewkesbury, United Kingdom

Introduction: Ricin is a toxin produced by the castor bean (*Ricinus communis*) and is a highly potent and lethal molecule. The active ricin consists of an A- and B-polypeptide chain coupled via a single S-S bond. The B-chain promotes binding and internalization into the cell. The A-chain inactivates the ribosome and inhibits protein synthesis. Timely and effective decontamination of the toxin from skin would contribute to the prevention of cross contamination and possible secondary routes of exposure. In the present study, the decontamination potential of the RSDL® (Reactive skin Decontamination Lotion) kit against ricin was evaluated.

Materials and Methods: Ricin was extracted and purified from castor beans and crude extract was characterized for quantity (RC DC™Protein Assay) and purity using SDS page. Quantification of purified ricin was performed using a sandwich ELISA assay.

RSDL lotion (Methoxy polyethylene glycol 550 (MPEG), KBDO and 2,3-Butanedione monoxime) and training-RSDL) were provided by Emergent BioSolutions.

The remaining ricin after incubation was quantified by ELISA, and remaining Ricin activity was studied in a cytotoxicity assay to determine the effects of RSDL lotion and its components.

The mechanism of inactivation was studied by using targeted LC-MS/MS analysis.

Results: The ELISA results revealed that treatment of purified ricin with the RSDL eliminated active toxin and coincided with a decreased toxicity demonstrated in cytotoxicity assay. It is demonstrated that KBDO in high concentration of MPEG or in 2M KOH solutions are critical for effective ricin inactivation. Structural modifications of ricin were evaluated using an LC-MS/MS based approach. The mass spectrometry results corresponded well with the ELISA data, and suggest that decrease in ricin activity by RSDL lotion was caused by an effect on the disulfide linkage between the ricin A and B chain.

Discussion and Conclusions: RSDL lotion was able to inactivate ricin, even after two minutes incubation. The mass spectrometry experiments suggest that the mechanism by which RSDL lotion inactivates ricin is by breaking of the disulfide linkage between the A- and B-chain, catalyzed by a combination of high pH and a nucleophile oximate (KBDO) in a matrix containing 90% MPEG. Additional experiments are needed to further elucidate the molecular mechanism of ricin inactivation by RSDL lotion.

IP16

Development of a multiparametric Spot-Immunoassay (SIA) for the detection of SARS-CoV-2 antibodies in COVID-19 patients

Philipp Sterna, Ariane Wenzel, Matthias Morgenstern, Abdullah Koskan, Lars Hoffmann, Sarah Richter, Renate Fischer, Klemens Löster, and Mathias Schlegel
Seramun Diagnostica GmbH, Heidesee, Germany

Background: The infection with SARS-CoV-2 (Family *Coronaviridae*) is known to be associated with the generation of variable levels of IgG, IgM, and IgA antibodies with virus neutralizing or non-neutralizing activity. The antibody levels change with time starting by induction of IgM and IgA antibodies followed by antibodies of IgG type. To improve the SARS-CoV-2 antibody serology with focus both on the course of the disease and vaccination, a multiparametric spot immunoassay (SIA) has been developed to detect IgG and IgA antibodies against the immunologically important SARS-CoV-2 nucleoprotein, spike protein and variants of it.

Methods: The *SeraSpot* Anti-SARS-CoV-2 IgG / IgA assay contains a total of four different SARS-CoV-2-specific antigens (nucleoprotein, spike protein, spike-S1 domain, spike S1-RBD) printed in array arrangement (spot array) on the bottom of the wells of 96well-microtitration plates. Binding of antigen-specific antibodies from human specimen is detected by horseradish peroxidase-(HRP)-labeled anti-human IgG and IgA antibodies and a chromogenic precipitating substrate solution. Developed arrays are imaged and evaluated with software support.

Results: The antibody profile of the samples from COVID-19 patients (n = 62) differed regarding the specificity for nucleoprotein and the spike protein antigens. Approx. 15% (IgG) and 20% (IgA) of the examined samples with nucleoprotein-specific immune response showed no reactivity with the spike protein antigens. The parallel occurrence of IgG and IgA antibodies was again distributed with 53% for nucleoprotein, 64% for S1-RBD, 46% for S1 domain and 44% for spike protein.

Furthermore, serum samples from Comirnaty/BNT162b2 (Biontech/Pfizer) vaccinated persons (n = 10) were screened for vaccine-induced antibodies. All samples showed high S1-RBD- and spike protein IgG antibody reactivities, but no or only weak IgA response and none nucleocapsid reactivities.

Conclusions: The *SeraSpot*® test system allows the simultaneous detection of nucleoprotein-, spike-, spike-S1- and S1-RBD-specific IgG or IgA antibodies from one sample in one well. This should not only enable the discrimination between active and past infections, but also be useful for antibody profiling or detection of possible protective antibodies. Since the *SeraSpot*® test allows a semi-quantification of SARS-CoV-2-specific antibodies it might support the serological testing in the context of vaccination control.

JP1

Murderous multicolored molds? Studies on detection and occurrence of toxinogenic micromycetes and mycotoxins in indoor environments

Wiebke Derz and Paul W. Elsinghorst
Central Institute of the Bundeswehr Medical Service Munich, Food Chemistry, Garching, Germany

Indoor mold is anchored in public awareness as a potential health hazard. For non-experts, a basic rule seems to apply: the more colorful, the worse. And black is bad. But is it that simple? In fact, inhalation of spores is associated with an increased prevalence of respiratory symptoms, allergies, asthma, and an impaired immune system and in addition to mycosis also mycotoxicosis can occur [1]. The biomass of a fungus consists of fine

fragments, which can be found floating around in indoor air. Fungi form secondary metabolites, among which are pigments giving the fungus its color, but also substances with toxic effects. These mycotoxins are present in high concentrations in spores and sclerotia of the fungi and can be absorbed when dust containing spores and sclerotia is inhaled [2]. But not all molds are equal and especially not equally bad. Toxin formation is influenced by various factors, and not every fungus does necessarily produce toxins [3]. To assess the risk of mold-infested indoor environments, cultural methods are commonly used. Yet, these methods completely disregard whether toxins are formed and, if so, which toxins are formed. The aim of the presented project is to collect data on fungal species by collection from air in different infested Bundeswehr premises and to match them with mycotoxins in airborne dust collected at the same time. After incubation of airborne germ samples fungi will be identified by microscopy and MALDI-TOF. Airborne particle samples will be extracted and screened for various secondary metabolites of fungi commonly found indoors using LC-MS/MS.

[1] World Health Organization, Regional Office for Europe: *WHO Guidelines for indoor air quality: dampness and mould.* 2009.

[2] Miller J. D., McMullin D. R.: *Fungal secondary metabolites as harmful indoor air contaminants: 10 years on.* Appl. Microbiol. Biotechnol. 98, 9953-66, 2014.

[3] Paterson R. R. M., Lima N.: *Toxicology of mycotoxins.* In Luch A.: *Molecular, Clinical and Environmental Toxicology: Volume 2: Clinical Toxicology.* Birkhäuser Basel, 2010.

JP2

Development of targeted mass spectrometry analysis for selected protein toxins

Miloslava B. Ďuráčová, Jana Klimentová, Alena M. Fučíková, Valeria Sheshko, and Zuzana Kročová
University of Defense, Faculty of Military Health Sciences, Hradec Králové, Czech Republic

Targeted proteomics recently proved to be a technique for the detection and absolute quantification of proteins not easily accessible to classical bottom-up approaches. Due to this, it has been considered as a high fidelity tool to detect potential warfare agents in wide spread kinds of biological and environmental matrices. In the present study, the quantitative proteomic assay for multiplex monitoring of bacterial and plant protein toxins with potential biowarfare significance was described. For the purpose of the method development protein standards of the toxins were first required. Where no suitable commercial standard could be obtained, the proteins were prepared in-house and the methods of their preparation form a substantial part of the results. Toxins from *Clostridium perfringens* were prepared as recombinant proteins in *E. coli* and isolated by a well-described affinity-tag protocol. The plant protein toxins - ricin and abrin -were isolated from the respective seeds. The obtained extracted proteins were in quality sufficient to the development of a targeted method. The remaining toxins (Botulinum neurotoxin type C, Cholera toxin, SA enterotoxin A, Shiga toxin subA, Viscumin (Beta-galactosidespecific lectin 1) were commercially available, however, their quality was not sufficient for further MS analysis and it was necessary to purify them. Protein standard of sufficient quality and amount is essential for the development of the targeted MS method. It was shown, that obtaining it may present the most complicated step of the workflow. A high-resolution mass spectrometer with a quadrupole-Orbitrap system operating in untargeted and target acquisition mode (parallel reaction monitoring) was utilized. The targeted MS method also utilized the addition of synthetic heavy peptides that allowed absolute quantification. For each protein, a set of two to eight unique peptides was selected. Peptides from the studied toxins can be thus analyzed together using a single inclusion list that can serve to monitor the potential presence of various protein contaminants in a single run.

JP3

Toxins standard preparation for target proteomic method

Valeria Sheshko, Miloslava Duracova, and Zuzana Krocova
University of Defence, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

The main aim of the present study was to develop, test and validate a targeted proteomic method for the detection *C. perfringens* protein toxins as a part of the biodefence project. *C. perfringens* toxins are considered to be potential biological weapons, especially the epsilon toxin which belongs to a group of the most powerful bacterial toxins. For the purpose of the method development protein standards of the toxins were first required. Where no suitable commercial standard could be obtained, the proteins were prepared in-house and the methods of their preparation form a substantial part of the present results. While production of selected *C. perfringens* toxins was highly desirable, attempts at their isolation from natural producers were problematic due to complexity of the protein samples and their low

production that severely limit the use of them for standard-grade toxins preparation for MS analysis without of enrichment with specific antibodies. Here we report an effective expression and purification approach for the alpha, beta, beta2, epsilon, iota a, and iota b proteins of *C. perfringens.* Genes of interests were amplified from *C. perfringens* chromosomal or plasmid DNA and cloned into pET28b+ expression vector to obtain his-tag fusion proteins. The recombinant proteins were expressed in *E. coli* and purified using immobilized metal-affinity chromatography. Identity and purity of the recombinant proteins were confirmed via electrophoresis and using data-dependent MS/MS analyses. Based on the previous DDA unique peptides were selected for each protein. The selection of representative peptides for targeted protein quantification is critical for targeted proteomics workflow development. For the *C. perfringens* toxins calibration, signals from labeled and unlabeled peptides eluting at the same time were acquired by the sequential acquisition method to avoid any overlap. The calibration curves were composed for each peptide in a range of concentrations from 0.635 fmol/μl to 1101 fmol/μl. Lower limits of detection and quantification were determined for each peptide.

JP4

Saxitoxin - an underestimated security threat

Jonathan S. Ullmann[1], Michael P.C. Fibinger[1], Arne Ficks[2], and Birgit Hülseweh[1]
1- Bundeswehr Research Institute for Protective Technologies and CBRN protection, B-Labor, Munster, Germany; 2- Bundeswehr Research Institute for Protective Technologies and CBRN protection, C-Labor, Munster, Germany

Saxitoxin (STX) is a highly poisonous low molecular weight non-peptide toxin that belongs to the group of paralytic shellfish poisoning (PSP) toxins. More specifically, it is a neurotoxin produced by selected species of cyanobacteria and marine dinoflagellates. The toxin blocks sodium channels of cell membranes, which subsequently may lead to paralysis and even death. STX can naturally accumulate in shellfish and it may be used on purpose to contaminate drinking water resources. Therefore, STX and its derivatives are of concern with regard to their potential use as a chemical warfare agent. Consequently, STX is a scheduled substance in the Annex of Chemicals of the Chemical Weapons Convention (CWC).

The reported LD_{50} (human) for oral administration of STX is 5.7 µg · kg $^{-1}$, while the value is ten times lower for intravenous administration. The inhalation toxicity for humans is estimated to be 5 mg·min·m^{-3} [1]. Alarmingly, STX-type toxins are not inactivated by common thermal decontamination methods (boiling/autoclaving). All these facts combined make STX a very dangerous and often underestimated security threat.

Different analytic methods for the detection and identification of STX-type toxins are available, for example enzyme-linked immunoassay (ELISA), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and LC with fluorescence detection (LC-FLD) with pre- or postcolumn oxidation, each method having its own advantages and disadvantages.

Commercially available ELISA, for instance, often lack the specificity for STX and the promised limit of detection of 0.1 ng/ml STX_{eq} [2] is often not achieved. LC-MS/MS can be used to distinguish between individual PSP derivatives without the need for additional chemical procedures (e.g. pre- or post-column oxidation), it is fairly sensitive with respect to the LOD (5 ng/ml) but requires extensive sample preparation. In contrast, HPLC-FLD has a much lower LOD (0.7 ng/ml), is very robust and quickly provides reliable and specific results. However, the method requires additional chemical processing like pre- or post-column oxidation.

In general, every sample matrix provides its own challenges for sample preparation and subsequent analysis. Highly individual processing steps, depending on the analytical method used are frequently required. Therefore, it is particularly valuable to take part in regular proficiency tests that allow the participants to improve their preparatory and analytical skills.

[1] Patocka, Jiri & Streda, Ladislav. (2002). Brief review of natural nonprotein neurotoxins. ASA newsletter. 02-2. 16-24. [2] Campbell, Katrina & Huet, Anne-Catherine & Charlier, Caroline & Higgins, Cowan & Delahaut, Philippe & Elliott, Christopher. (2009). Comparison of ELISA and SPR biosensor technology for the detection of paralytic shellfish poisoning toxins. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 877. 4079-89. 10.1016/j.jchromb.2009.10.023

JP5

Validation of methods for inactivation of equipment and clothing contaminated with ricin

Christine Goers[1], Julia Schalhorst[1], Andreas Bachmann[2], Nico Fortwengel[1], and Henri Derschum[1]

1- CBRN Defense Safety and Environmental Protection School (CDSEP School) , Biology Section, Sonthofen, Germany; 2- Defense Safety and Environmental Protection School (CDSEP School), Biology Section, Sonthofen, Germany

Ricin is a potent protein toxin that can be readily extracted from the seeds of the castor bean plant, *Ricinus communis*. The two-chained glycoprotein phytotoxin specifically and irreversibly inactivates ribosomes, promoting cell death by inhibiting protein synthesis. Just one molecule is enough to cause the death of a cell. Well known for its potent toxic effect and ubiquitous availability of the plant, ricin could be misused easily for harmful purposes - such as a bioterrorism event.

Although literature cites different detoxification methods using physical treatment or chemical processes, available inactivation conditions for garment or personal equipment are not characterized in detail.

For a practical application, we describe concatenation of laboratory scale experiments ending with an upscaled inactivation process of potentially contaminated garments and personal equipment in an actual case.

Two scientifically proven (common) methods of inactivation were each validated for subsequent use in combination on materials contaminated with ricin.

Toxin recovery, validation of inactivation and upscaling for an actual inactivation:

Prior to experimental validation of inactivation efficiency, recovery rates for residual toxin on different surfaces were defined to account for loss of potentially active toxin during the processes. This was done to ensure that no medically significant quantities of the powder-like toxin were overseen, especially when hypothetically evading the inactivation process.

Residual toxin levels were subsequently quantified using an in-house ELISA as recommended by the national Centre for Biological Threats and Special Pathogens at the Robert-Koch-Institute (RKI).

Validation of inactivation: During inactivation tests, garment and solid material samples (n=10) were each contaminated with 150 ng ricin standard. After a 5-day exposure period, these samples were subjected to inactivation procedures. Recovery rates for toxin were defined using the same samples beforehand. The quality of the recovery is the decisive parameter for assessing the reliability of the determination of the inactivation success in the subsequent laboratory tests. The recovery rate averaged over all four tests was 97% +/-5.1%, which was considered appropriate for continuation of the validation experiments.

For safety reasons we decided to concatenate two individual methods of inactivation, one being chemical degradation - alkaline denaturation, and one additional physical treatment - temperature. This allowed for concentrations, incubation times and physical parameters that were not too harsh on provided garment and personal equipment, but to a standard sufficient to allow unrestricted transportation, maintenance, employment and disposal. Therefore, after positive evaluation of quality assurance samples, we were able to hand back equipment and garment for further use.

KP1

Adaption of peroxyacetic acid (PAA) aerosol-based disinfection protocols to field conditions

Hendrik A. Scheinemann[1], Jan Schinköthe[2], Jen P. Teifke[1], and Sven Reiche[1]
1- Friedrich-Loeffler-Institut, Department of Experimental Animal Facilities and Biorisk Management (ATB), Greifswald, Germany; 2- University of Leipzig, Institute for Veterinary Pathology, Leipzig, Germany

Background: After establishing suitable aerosol-based disinfection protocols in high-containment facilities, presented in 2018, we started to investigate the field suitability of this method. Actual, this question is of pivotal importance caused by the tense situation of the spreading African swine fever virus in Europe as well as quick response after bio attacks. Thereby, the influence of multiple different climate conditions on the disinfection efficacy has to be evaluated.

Materials and Methods: Spore forming bacteria, mycobacteria and mould spores were inoculated on stainless steel carriers according to quantitative carrier testing protocols (DVG, RKI). The carriers were placed at different locations within a provisory sealed radio shelter (20.56 m^3) that was aerosolized with ultrafine particles (7.5 "m) of a 100 ml solution containing 1.3 % PAA. After an incubation time up to 60 min and 1 h aeration phase, the microorganisms were recovered and the inactivation efficacy was determined as log extsubscript10 reduction. Remaining germ titers were determined by using standard titration techniques and the automated quality control device "BioLumix" in parallel. The tenacity of the used surrogates where compared to BSL 3 strains used in bio terroristic attacks or from recent outbreaks.

Results: The adapted and validated protocols resulted in a $\geq$ 4 log extsubscript10 reduction of all

tested microorganisms. An incubation time of 60 minutes was adequate for low temperature ranges (10 to 0°C). Warmer surfaces ($\Delta T \geq 4°C$) are protected by boundary layers independent of the absolute temperature and lead to reduced inactivation efficacies. The tested automated device is suitable to determine remaining germ titers within 19 h. The used surrogates had a similar or even higher tenacity compared to the BSL3 pathogens.

Conclusion: The PAA aerosol-based disinfection method is robust and highly effective in rendering contaminated areas or devices safe to handle. Suitable automated devices will simplify the analyses and significantly shorten readout times.

KP2

Revision of EN 12469 - New requirements to microbiological safety cabinets in Europe?

Thomas Hinrichs[1], Ralf Wörl[2], and Michael Klein[3]
1- Berner International GmbH, General Management, Elmshorn, Germany; 2- Berner International GmbH, Productmanagement, Elmshorn, Germany; 3- Berner International GmbH, Head of R&D, Elmshorn, Germany

Microbiological safety cabinets (MSCs) of class I, II and III are the most important laboratory devices to protect the user and the environment from risks associated with the work with microorganisms and other potentially hazardous biological agents. Additionally, MSCs provide product protection for sensitive biological material and avoid cross-contamination in susceptible experiments.

The European standard EN 12469 describes essential performance criteria for MSCs, but the last version became effective already in May 2000. More than 20 years have naturally led to changes in the state of the art, so it has been long since the time for a revision. Work groups in the German national committee (DIN-Standard committee for laboratory devices and laboratory facilities, FNLa; NA 055-02-04 AA) and in the superordinate European Committee (CEN/TC 332/WG8 „Safety cabinets and isolators“) have brought the future EN 12469 to a complex working paper in the past months with promising new requirements.

The purpose of this presentation is to give an overview about the upcoming and most important changes in the requirements to MSCs. A main topic will be the enhanced performance requirement which should help to ensure high protection levels even under critical working conditions.

KP3

Bioaerosol chamber for directed experiments with *Legionella pneumophila*

Lena Heining[1], Michael Seidel[1], Laura Welp[2], and Achim Hugo[2]
1- Technische Universität München, Institute of Hydrochemistry, Munich, Germany; 2- Institut für Energie- und Umwelttechnik, Duisburg, Germany

Legionella outbreaks have occurred repeatedly in cooling towers in recent years. So far, there are only guidelines for monitoring *Legionella* concentrations in water but not in aerosols. The relationship between the concentration in water and in aerosols has not yet been fully investigated. This is one reason why it is important to establish facilities, where targeted bioaerosol studies can be carried out without risk. The problem with the analysis of *Legionella* is that it can enter a viable but not culturable (VBNC) state. To avoid underdetermination and long analysis time of *Legionella* through cultivation, it is necessary to develop culture-independent methods.

Our aim is the establishment of a bioaerosol chamber, which can be manifoldly utilized for studies on bioaerosols. Therefore, a glovebox was modified to a safety workbench, which works with negative pressure, so that there is no occurrence of uncontrolled release of bioaerosols. Further, the openings for incoming and outgoing air are equipped with HEPA-filters.

In the bioaerosol chamber aerosol samplers are characterized with regard to recovery and survival of bacteria during sampling. For this purpose, we nebulize bacteria suspensions with a Pari LC nebulizer and the sampling occurs with three different types of aerosol samplers. The impingement (AGI 30) is the common used method and we want to compare it firstly with a cyclone sampler, the Coriolis ". Secondly, the Next Generation Impactor is used for the achievement of a size-dependent determination of *L. pneumophila* in moist droplets.

As mentioned, it is important to develop culture-independent strategies for the analysis of *L. pneumophila*. With an antibody based chemiluminescence sandwich immunoassay a sero- and subtypification on a microarray platform is possible. For molecular biological methods, an integrity-qPCR as well as a heterogeneous asymmetric recombinase polymerase amplification will be performed for distinction between living and dead cells. In addition, all of these analytical strategies are compared to cultivation.

In the further procedure, a laboratory bioaerosol incubator is installed in the chamber, in which a biofilm with *Legionella* is cultivated. On the one hand, this will provide information on the distribu-

tion of *Legionella* between the water and air phases. On the other hand, it offers possibilities to carry out investigations on the treatment of contaminated biofilms and the possible release of *Legionella*.

KP4
Plasma Decontamination Techniques

Kathrin Kluge and Nikolaus Schneider
Bundeswehr Research Institute for Protective Technologies and CBRN Protection, Decontamination, 29633 Munster, Germany

In general, the amount of sensitive equipment used for military applications such as electronics, optics, optronics and computers, has increased significantly in recent years. At the same time, procurement favours COTS/MOTS instead of custom-made products whenever it seems acceptable. Such equipment, however, may not withstand established decontamination procedures based on high temperatures or wet chemical treatment. We are continually seeking more efficient, environmentally friendly, gentle and logistically convenient decontamination solutions in order to further improve existing capabilities of the Bundeswehr. Plasma based decontamination technology is a very promising candidate for the armed forces since it has been already established in medical applications, in the food industry and for material and surface treatments. Plasma is an ionised gas created by either very high temperatures or electronic discharges on specific electrodes. It consists of reactive species such as electrons, ions, excited molecules and radicals, which are able to degrade biological or chemical agents. Herein we compare and evaluate the efficiency and suitability of different plasma based technologies for military purposes. Cold Atmospheric Plasma (CAP) operates in ambient air without the need for specific gases. Therefore, it has logistical advantages, however, CAP devices are commercially unavailable in application size and the technical robustness of our 100 L research prototype is poor. In contrast, vacuum plasma chambers are commercially available on application scale but they do require an elaborate vacuum environment consisting of pumps and an airtight enclosure, the operation of which may be cumbersome in field operations. In the context of plasma technology we also investigate a newly developed (at the Leibniz Institute for Plasma Science and Technology) disinfectant called Sporosan® for wet chemical decontamination processes, whose substrate chemicals can be generated using plasma.

KP5
Respiratory protection against biohazards exemplified by the COVID-19 pandemic

Jana Wiemann, Friedrich Hesse, and Thomas Dawert
Bundeswehr Research Institute for Protective Technologies and CBRN Protection, 410 - NBC-Individual- and Collective Protection, Munster, Germany

In the context of the COVID-19 pandemic, respiratory protection has become commonplace to suppress the spread of aerosol-borne infectious particles.

The protective effect of face masks depends on two aspects: the efficiency of the filter media and the tight fit on the face. Whereas the filter efficiency is a material constant for a face mask, the fit is highly individual depending on face dimensions and the adaptability of the mask. Already small leaks in the sealing line can significantly reduce the overall protective effect of the face mask. According to the international standard EN 149 (Respiratory protective devices - Filtering half masks to protect against particles), this aspect is taken into account by the complex measurement of the total inward leakage (TIL) based on a comparison of the mass concentration of a test aerosol inside and outside the mask. However, the mass concentration as a test criterion for the evaluation of the protective effect against microorganisms is not as relevant as for chemical or physical threats.

With regard to biological hazards, the particle-number density is more significant, thus alternative test methods for the evaluation of the total inward leakage have been assayed regarding their applicability and validity. The results of two different test setups (WIS field mask tester and PortaCount®) have been compared by the examination of different mask types. Additionally, measurements have been performed focusing on the determination of the influence of small, defined leakages on the overall protective effect of face masks. Furthermore, the size of the particles that penetrate the filter medium and the size of the particles that pass the leaks of the sealing line were analysed and compared.

LP1
[Contribution withdrawn]

LP2
Discussion on the COVID-19 Roots from the Aspect of Biological weapon

Levent Kenar
University of Health Sciences, CBRN Department,

Ankara, Turkey

COVID-19 outbreak which appeared first as atypical pneumonia cases with unknown etiology in Wuhan city of China in last days of 2019 according to official reports and spread to first Iran and then through entire European countries including mainly Italy, UK and United States of America has emerged also in our country in mid-March 2019 and been on the agenda in our routine lives with extending quarantine and isolation measures. Since the epidemics broke out, scientists have claimed many considerations and findings about the origin and evolvement of coronavirus and tried to find answers whether the agent is a kind of biological weapon manufactured in the laboratory. Hypotheses and antihypotheses on whether the virus is nature-made as the result of natural selection or human- made still remain on the agenda of the world. Despite the bans indicated in international conventions, possibility of being a bioweapon or revealing the birth place of coronavirus will lead the type of our expected future. So, this presentation explains the concept of biowarfare, main characteristics of biological weapons, the exact place of coronavirus in the context of components of biodefense and will evaluate the virus from the aspect of biological weapon.

MP1

[Contribution withdrawn]

MP2

Identification of novel chemical scaffolds as anti-DENV compounds.

Salvatore Ferla[1], Andrea Brancale[2], Silvia D'andrea[2], Nicolo Santi[2], and Giulio Nannetti[2]
1- Swansea University, Swansea, United Kingdom; 2- Cardiff University, Cardiff, United Kingdom

Dengue virus (DENV) is a mosquito-borne, (+)RNA, virus that belongs to the Flaviviridae family, and it is responsible for a disease that can range from a mild, subclinical manifestation, to a severe flu-like illness. A severe form of dengue, dengue haemorrhagic fever, can also occur, presenting a higher risk of death. The cases of dengue have been constantly, and rapidly, multiplying: the WHO reported that the DENV infections have increased by more than 8 fold in the last twenty years, with 5.2 million cases reported in 2019. Despite this, no current specific antiviral therapy has yet been approved, while vaccines still have some limitations.

Our work aims to identify novel small molecules as specific anti-DENV compounds. In particular, in this presentation, we describe our initial efforts in the identification of novel chemical scaffolds that could inhibit the DENV RNA-dependent RNA-polymerase (RdRp), an essential enzyme in the viral replication process. Based on the available structural information of the RdRp, we have used a structure-based computational approach to screen a library of >5million commercially available compounds *in silico*. The molecular docking results obtained were ranked applying a combination of different scoring functions, in a general consensus score. The most promising compounds were then purchased and tested in a NS5 RdRp enzymatic activity assay, and in a cell-based antiviral assay.

Interestingly, a small number of diverse compounds showed inhibitory activity at a micromolar range. Although more effort is required to optimise these hits, they still represent an excellent starting point for the development of novel therapeutic antivirals that specifically target DENV.

MP3

Evaluation of Neutralizing Antibodies Against SARS-CoV-2 in a vaccinated population: comparison between a commercially available kit and PRNT

Maria S. Lia, Elisa Regalbuto, Donatella Amatori, Alessandra Amoroso, Andrea Ciammaruconi, Silvia Fillo, Giovanni Faggioni, Florigio Lista, and Riccardo De Santis
Army Medical Center, Scientific Department, Rome, Italy

The SARS-CoV2 pandemic has spread around the world causing extraordinary public health challenge. In this unstable scenario, serological tests represent promising tools for monitoring seroconversion in both post-vaccinated population and patients recovered from the disease. Currently, Plaque Reduction Neutralization Test (PRNT) represents the gold standard for the neutralising antibody testing. Nevertheless, this assay has several limitations as required access to biosafety level 3 (BSL3) with highly trained staff and overall time of execution, resulting very time and cost consuming. For this reason, reliable high throughput automated assays are urgently needed. The aim of this work was the evaluation of neutralizing antibodies against SARS-CoV-2 in a vaccinated population comparing the results obtained through the PRNT method and the automated platform LIAISON® SARS-CoV-2 TrimericS IgG test (Diasorin).

The study population consist of 360 immunocompetent health workers of the Army Medical Center (Policlinico Militare of Rome "Celio"), among which

263 were male (average age of 44.3) and 97 female (average age of 40.5), vaccinated with Comirnaty (Pfitzer/Biontech), from January to March 2021. For each patient three different samples were collected at three different time points (T0=before vaccination; T1=21 days after first injection; T2= 21 a days after second injection). A number of 1,080 samples were collected for a total of 3,240 data analysed.

The results obtained from this seroepidemiological study evidenced a very good accordance between the compared techniques (p-value: 0.77). In order to evaluate the analytical and clinical performance (sensitivity and specificity) of LIASON SARS CoV-2 TrimericS IgG test a ROC curve was performed, resulting in a remarkable area under the curve (AUC) (0.97).

In conclusion, the obtained results validated the reliability of the commercially available tool tested, allowing to obtain useful information to monitoring the strength and duration of immunity after vaccination. Further studies with the same approach are still ongoing on infected subjects evaluating the residual antibody response.

MP4

Evaluation of humoral and cellular immunity after vaccination against SARS-CoV-2

Katharina Müller, Philip Girl, Enrico Georgi, Gerhard Dobler, Roman Woelfel, and Heiner von Buttlar
Bundeswehr Institute of Microbiology, Munich, Germany

Since the end of 2019 the pandemic spread of SARS-CoV2 challenges health systems world wide and let to thousands of related fatalities. The most efficient countermeasure against the virus is vaccination. Vaccination campaigns reduced the number of infected patients and especially the occurence of severe diesease progression, taking pressure from the local health systems. The induction of SARS-CoV-2-spike specific, virus neutralising antibodies is one explanation for vaccine efficacy. This is clearly emphasized by the therapeutic success of plasma donation to severly affected patients. Nevertheless, T-cell mediated immune mechansims have been shown to be of great importance in the fighting of viral diseases, i.e. for closely related MERS-CoV. Therefore, we offered the analysis of humoral and cell mediated immunity after vaccination against SARS-CoV-2. Besides the detection of SARS-CoV-2 spike specific antibodies even the neutralising capacity of the sera against the virus was measured. Moreover, using commercially availabel stimulation matrices (Quiagen) the antigen specific cytokine secretion of T cells in a whole blood stimulation assay was assessed. Data from different vaccination schedules could be compared.

Here we show the feasability to analyse vaccination success not only by the detection of antigen specific antibodies, but also by testing T-cell responses to specific re-stimulation. The usage of whole blood stimulation method turned out to be a robust tool that makes T-cell analysis feasable even in situations where blood sampling and further processing is interupted for several hours due to the need of transportation.

MP5

Two-stage development of a recombinant Modified Vaccinia virus Ankara expressing Lassa virus glycoprotein

Alina Tscherne[1], Georgia Kalodimou[1], Jan H. Schwarz[1], Sylvia Jany[1], Astrid Freudenstein[1], Asisa Volz[2], and Gerd Sutter[1]
1- Division of Virology, Department of Veterinary Sciences, Munich, Germany; 2- Institute of Virology, University of Veterinary Medicine, Hannover, Germany

Lassa virus (LASV), causative agent of the severe infectious disease Lassa fever (LF), is endemic in several countries in West Africa. Up to 300,000 cases are confirmed every year and an expansion of the virus to non-endemic regions within the last years highlights the importance of developing effective vaccines and treatments. Although several DNA and RNA-based vaccines are tested in preclinical and clinical research, no LASV-specific vaccines are licensed for prevention of LF by now.

Modified Vaccinia virus Ankara (MVA) is a well characterized orthopoxvirus and vaccine strain unable to productively replicate in cells of human origin. Due to an established record of clinical safety and its capacity to efficiently deliver various recombinant proteins as antigens, MVA is a promising tool to create candidate vector vaccines against emerging infections. Here, we describe the generation and characterization of a recombinant MVA expressing LASV glycoprotein (MVA-LASV-GP) using our vector technology platform for the swift development of candidate MVA vaccines compliant with requirements for clinical research. Clonal MVA-LASV-GP was isolated using a two-step plaque purification process monitoring for transient co-production of LASV-GP and the red fluorescent protein mCherry. Genetic stability, stable expression of the viral antigen and the growth characteristics of recombinant MVA-LASV-GP make it a suitable candidate for

further testing in preclinical models.

MP6

Immunogenicity and efficacy of the COVID-19 candidate vector vaccine MVA-SARS-2-S in preclinical vaccination

Alina Tscherne[1], Jan H. Schwarz[1], Cornelius Rohde[2], Alexandra Kupke[2], Georgia Kalodimou[1], Leonard Limpinsel[1], Nisreen M.A. Okba[3], Berislav Bošnjak[4], Inga Sandrock[4], Sandro Halwe[2], Lucie Sauerhering[2], Katrin Brosinski[1], Nan Liangliang[1], Elke Duell[1], Sylvia Jany[1], Astrid Freudenstein[1], Jörg Schmidt[2], Anke Werner[2], Michelle Gellhorn Serra[2], Wolfgang Guggemos[5], Michael Seilmaier[5], Clemens Wendtner[5], Reinhold Förster[4], Bart L. Haagmans[3], Stephan Becker[2], Gerd Sutter[1], and Asisa Volz[6]
1- Division of Virology, Department of Veterinary Sciences, LMU Munich, Munich, Germany; 2- Institute of Virology, Philipps University Marburg, Marburg, Germany; 3- Department of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands; 4- Institute of Immunology, Hannover Medical School, Hannover, Germany; 5- Klinikum München-Schwabing, Munich, Germany; 6- Institute of Virology, University of Veterinary Medicine, Hannover, Germany

The severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) has emerged as the infectious agent causing the pandemic coronavirus disease 2019 (COVID-19). Previously, we reached clinical evaluation with our vector vaccine based on vaccinia virus MVA against the Middle East respiratory syndrome coronavirus (MERS-CoV), causing an infection similar to SARS and COVID-19. Here, we describe the construction and preclinical characterization of a recombinant MVA expressing full-length SARS-CoV-2 spike (S) protein (MVA-SARS-2-S). Genetic stability and growth characteristics of MVA-SARS-2-S, plus its robust synthesis of S antigen, make it a suitable candidate vaccine for industrial scale production. Vaccinated mice produced S antigen-specific CD8+ T cells and serum antibodies binding to S glycoprotein that neutralized SARS-CoV 2. Prime-boost vaccination with MVA-SARS-2-S protected mice sensitized with a human ACE2-expressing adenovirus from SARS-CoV 2 infection. MVA-SARS-2-S is currently being investigated in a phase I clinical trial as aspirant for developing a safe and efficacious vaccine against COVID-19.

MP7

PEGylation of enrofloxacin reduces minimum inhibitory concentrations and hepatoxic effects in rats

Vasyl Vlizlo[1], Dmytro Ostapiv[2], Volodymyr Samaryk[3], Volodymyr Stybel[4], Mariya Kozak[2], Boris Stegniy[5], Oksana Zelenina[6], and Iryna Dron[3]
1- Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Department of Internal Animal Diseases and Clinical Diagnostics, Lviv, Ukraine; 2- Institute of Animal Biology, NAAS of Ukraine, Laboratory of molecular biology and clinical biochemistry, Lviv, Ukraine; 3- Lviv Polytechnic National University, Organic Chemistry, Lviv, Ukraine; 4- Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Lviv, Ukraine; 5- National scientific center «Institute of Experimental and Clinical Veterinary Medicine, Charkiv, Ukraine; 6- Odesa State Agrarian University, Odesa, Ukraine

Polyethylene glycol (PEG) as a drug carrier is promising for the development of current and new antibiotic compounds via their modification. The process of chemical attachment of PEG to a native drug molecule is called PEGylation. The aim of the study was to PEGylate the antibiotic enrofloxacin to increase its antimicrobial activity and improve therapeutic efficacy. PEGylation of enrofloxacin was performed using polyethylene glycol (nanopolymer PEG-400). A *high-performance liquid chromatography* showed 98-99% purity of PEGylated antibiotic enrofloxacin. To study the antimicrobial activity of PEGylated enrofloxacin and enrofloxacin, the minimum inhibitory concentrations (MIC) were determined using reference museum strains of *Escherichia coli* ATCC 11105 and *Staphylococcus aureus* ATCC 6538P. It was found that MIC of enrofloxacin against *E. coli* was 0.31 µg/ml and MIC of the PEGylated enrofloxacin was 0.15 µg/ml. The MICs of both of traditional enrofloxacin and PEGylated enrofloxacin against *S. aureus* were the same (0.31 µg/ml). Four groups of rats (control and three experimental) were formed to study the hepatotoxicity of the enrofloxacin in traditional and PEGylated forms. The animals of control group were injected intramuscularly with saline in a volume of 0.03 ml. Rats of the 1st experimental group were injected intramuscularly with enrofloxacin in a volume of 0.03 ml, 2nd - 0.03 ml of PEG 400, 3rd - 0.03 ml of PEGylated enrofloxacin. Biochemical studies of animal blood (aspartataminotransferase, alaninaminotransferase, alkaline phosphatase and gamma-glutamyltranspeptidase, cholesterol, total protein and albumin levels) and histological analysis of the liver indicated that the PEGylated enrofloxacin had lower hepatotoxicity than traditional enrofloxacin. Thus, PEGylation of enrofloxacin improved the antimicrobial properties of the antibi-

otic and has a lower hepatotoxic effect on animals compared to the traditional form of the drug. In the future, we plan to test the PEGylated enrofloxacin for the treatment of animals.

MP8

Assessment of knowledge and awareness of the need for vaccination against COVID 19 among employees of the Military Institute of Medicine and people from outside the Military Institute of Medicine vaccinated at a hospital vaccination point

Karol Warda[1] and Agnieszka Wozniak-kosek[2]
1- Military Institute of Medicine, Higiene and Epidemiology, Warsaw, Poland; 2- Military Institute of Medicine, Laboratory Diagnostics, Warsaw, Poland

The global COVID-19 immunization guidelines recommend that people over the age of 12 should be extensively vaccinated. In Poland, it is a voluntary vaccination, financed from public funds. The order of vaccination was determined by the Ministry of Health and described in the "National Program for Vaccination against COVID-19". The medics were included in the "0" group, i.e. "vaccinated as first". The aim of the study is to get to know the basic level of knowledge, opinions and declared behaviours regarding COVID-19 among various age and professional groups of the Polish population related primarily to health protection. The research was carried out using the diagnostic survey method during the vaccination campaign against COVID-19 carried out at the vaccination point of the Military Institute of Medicine, having prepared for this purpose 20 substantive closed questions. Respondents were vaccinated with all types of vaccines registered in the EU. The target group were medical and non-medical workers receiving vaccinations at the Military Institute of Medicine. In the period of January-March 2021, 4,545 questionnaires were obtained from those who vaccinated, 73% of whom are women. The vast majority of respondents live in cities, only 15.6% in rural areas. The study shows that 16.7% of those vaccinated were infected in the past with SARS-COV-2. In the entire analyzed cohort, 77.1% stated that they had been vaccinated against tuberculosis, but 71.4% of respondents did not declare their willingness to vaccinate against influenza in this epidemic season. Respondents most often look for knowledge about COVID-19 and its prevention on official WHO websites, government websites or in conversations with a primary health care physician. Television and various types of communicators are considered unreliable by the majority of respondents. Contact with a doctor definitely translates into increased awareness of the need to vaccinate also in the family circle 84.9%. Knowledge of complications after Covid-19 disease is 86.9% in the entire cohort. It is still necessary to conduct general educational campaigns to popularize knowledge about the risks, complications and methods of treatment, especially in the environment of people undecided about accepting vaccination against COVID-19. Perhaps a good solution as an incentive for mass vaccination will be to organize various types of festivals with lotteries and prizes for the undecided.

MP9

Transmission of a novel antimicrobial resistant strain of *Yersinia pestis* during a pneumonic plague outbreak

V Andrianaivoarimanana[1], D M. Wagner[2], D N. Birdsell[2], B Nicolay[2], F Rakotoarimanana[1], L N. Randriantseheno[1], A J. Vogler[2], J W. Sahl[2], C M. Hall[2], N Somprasong[1], S Cauchemez[2], H P. Schweizer[2], H Razafimandimby[1], C Rogier[1], and M Rajerison[1]
1- Institut Pasteur de Madagascar, Plague Unit, Antananarivo, Madagascar; 2- Northern Arizona University, Pathogen & Microbiome Institute, Flagstaff, USA

Pneumonic plague (PP), caused by *Yersinia pestis*, is the most feared clinical form due to its rapid lethality and potential to cause outbreaks. PP outbreaks are now rare due to antimicrobial therapy.

A PP outbreak in Madagascar involving transmission of a *Y. pestis* strain resistant to streptomycin, the recommended first-line treatment, was retrospectively characterized using epidemiology, clinical diagnostics, molecular characterization, and animal studies.

The outbreak occurred in February 2013 in the Faratsiho district of Madagascar and involved 22 cases, including three untreated fatalities. The 19 other cases participated in funeral practices for the fatal cases and fully recovered after combination antimicrobial therapy. The *Y. pestis* strain that circulated during this outbreak is resistant to streptomycin resulting from a point mutation in the 30S ribosomal protein S12 (*rpsL*) gene, the first description of antimicrobial resistance (AMR) arising in *Y. pestis* due to chromosomal mutation. This same mutation produces streptomycin resistance in an unrelated *Y. pestis* strain isolated from another location in 1987, documenting this mutation has occurred independently multiple times in *Y. pestis* strains from Madagascar. The mutation has no detectable impact on fitness or virulence, suggesting *Y. pestis* strains possessing it could persist in the

environment.

Novel AMR strains of *Y. pestis* continue to arise in Madagascar and can be transmitted during PP outbreaks. The fatality rate associated with this outbreak would likely have been higher if attending physicians had not utilized a combination treatment protocol: intramuscular streptomycin followed by oral co-trimoxazole.

NP1

Rodents in Kazakhstan are a natural reservoir of *Rickettsia*

Edith Wagner[1], Nurkeldi Turebekov[2], Nur Tukhanova[2], Anna Shin[2], Christina Ehrhardt[3], Lukas Peintner[1], and Sandra Essbauer[1]
1- Bundeswehr Institute of Microbiology, Virology and Intracellular Agents, Munich, Germany; 2- Aikimbayev's National Scientific Center for Especially Dangerous Infections, Almaty, Kazakhstan; 3- Institute of Medical Microbiology Jena University Hospital, Section of Experimental Virology, Jena, Germany

Records on the distribution of Rickettsia in the Central Asia are incomplete, including the area of the Republic of Kazakhstan. First description of Rickettsioses in the Kazakh city of Almaty was made in the 1950ies and since 1995 tick-borne Rickettsioses are officially registered. Currently, endemic areas are North Kazakhstan, Pavlodar, East Kazakhstan and Kyzylorda. In the previous 25 years, 4,836 human cases of tick-borne rickettsioses were registered.

A previous study reported the presence of murine/endemic typhus in Almaty and Kyzylorda Oblasts in arthropod vectors. However, wild animals such as rodents have to act as natural reservoirs for *Rickettsia* to maintain a beneficial lifecycle. Such data on the natural lifecycle of *Rickettsia* are available for Western countries, but - to our knowledge - no data are available in Kazakhstan so far.

Here we present for the first time data on *Rickettsia spp.* isolated from rodent ears in two oblasts in Kazakhstan.

Collected rodents (n=624) from Almaty Oblast and West Kazakhstan were screened for an infection by *Rickettsia spp.* DNA was extracted from ear pinna and screened by real-time PCR targeting the pan-rickettsial citrate synthase gene (gltA). From all analysed rodents 43 (7%) were positive for Rickettsia. A subsequent sequencing of isolated Rickettsia DNA revealed the same heritage as observed in ticks.

In summary, this study proved that rodents in Kazakhstan serve as a natural reservoir of Rickettsia.

NP2

Adapting biosafety and biosecurity teaching and learning in times of COVID-19 in Kazakhstan

Lukas Peintner[1], Franziska Stoek[2], Ansgar Schulz[2], Olga Spaiser[3], Janine Dywicki[4], Edith Wagner[1], Sulushash Zhumabayeva[5], Sandra Essbauer[1], and Dewi I. Puradiredja[6]
1- Bundeswehr Institute of Microbiology, Department Virology and Intracellular Agents, Munich, Germany; 2- Friedrich-Loeffler-Institut, Institute of Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany; 3- Deutsche Gesellschaft für Internationale Zusammenarbeit GmbH, Berlin, Germany; 4- Robert Koch Institut, Zentrum für Internationalen Gesundheitsschutz (ZIG3) - Notfallvorsorge und Einsatzunterstützung, Berlin, Germany; 5- Deutsche Gesellschaft für Internationale Zusammenarbeit GmbH, Almaty, Kazakhstan; 6- Bernhard-Nocht-Institute for Tropical Medicine, Department of Infectious Disease Epidemiology, Hamburg, Germany

The 2020-2021 COVID-19 pandemic hit international biosafety and biosecurity capacity-strengthening projects in an unprecedented way. Within the German-Kazakh Network for Biosafety and Biosecurity key activities, such as in-person networking, field visits and trainings, were not possible due to travel restrictions. To maintain a minimum of communication and scientific exchange, alternative modes of communication had to be identified and initiated. The German-Kazakh Network for Biosafety and Biosecurity is part of the German Federal Foreign Office funded German Biosecurity Programme that jointly operates the German Online Platform for Biosafety and Biosecurity (GO4BSB), a digital platform available to all members of the German initiative. During the pandemic, this platform became the central information hub for German training initiatives in Kazakhstan. Here we present two initiatives that demonstrate how trainings in biosafety and biosecurity can be adapted within the restrictive context of the COVID-19 pandemic.

The onset of the pandemic saw a surge in the demand for qualified lab technicians to conduct SARS-CoV-2 PCR screening from swabs. To train technicians in the fundamentals of biosafety and methods on COVID-19 diagnostics, GO4BSB initiated a self-study course within its COVID-19 Digital Initiative. Seven modules teach in three languages (English, French and Russian) the basics on how to safely handle swabs, isolate viral RNA, and conduct WHO

approved PCR screening. Progress is monitored by pre- and post-tests and regular self-assessment tests throughout the learning experience.

Besides the self-study modules, we offered a facilitated virtual training on biosafety concerns with a local trainer, who held online-conference-based classes backed by learning material shared via the GO4BSB platform. Here, learning progress of the student was being monitored by pre- and post-tests.

In summary, due to the pandemic, teaching activities were forced to switch to digital and virtual tools. After overcoming initial challenges related to technical issues and acceptance, these two initiatives show how teaching and learning can represent effective alternatives to in-person trainings, at least for theory-based topics.

NP3

The role of biorisk department in improving biosafety and biosecurity in departments dealing with infectious disease

Siza O. Mukhtar, Elameen A. Mohammed, Huyam O. Mohamed ElHassan, and Sanaa M. Babikir
National public health laboratory, Biorisk department, Khartoum, Sudan

Introduction: Sudan is large country in Africa suffering from many challenges in the field of infectious disease and outbreaks which is major threats affecting the world, therefore National public health laboratory (NPHL) in the Sudanese ministry of health dealing with wide range of pathogens like COVID-19, influenza, Hepatitis B virus, textit-Mycobacterium tuberculosis etc. which need implementing effective Biorisk management system (BRM). The importance of building strong biosafety and biosecurity system it is critical needs to safe people whom dealing with highly pathogenic agents.

The biosafety and biosecurity department established in 2014 at NPHL for managing risk all over Sudan laboratories. NPHL Adopted CWA 15793:2011 as standard for the biorisk management system for 17 departments through the fixed strong system contain polices, principles, documents, technologies and practice and training with Top management commitment.

Methodology: (1) Laboratory assessment: Laboratory assessment by using gap analysis for implementation of CWA 15793:2011 tool. Adapted the gap analysis for implementation of CWA 15793:2011 tool to the Sudanese laboratory situation (level 2). Revise the process by MHH & RKI. (2) Audit check list CWA 15793 National public health laboratory (NPHL) Sudan: Developed by experts from Hannover Medical school and Robert Koch Institute.

Results:

- Number of measures were assigned on the basis of 16 elements CWA 15793 - 2011
- Institutional policy in place
- Risk assessment procedure well established
- Good documentation and communication
- Good microbiological practice and procedure improved
- Urgently there is need for implementing biosecurity measures

Conclusion: Biorisk department at NPHL have prominent role in protecting laboratory staff dealing with highly infectious agents.

NP4

Strengthening the Performance of the Biorisk Management Systemn through internal Biorisk Visits

Murat Kaplan, Gülnur Kalaycı, Zahide Dilik, Gülçin Erdal, Fethiye Çöven, Murat Çetin, and Ozhan Türkyılmaz
İzmir/Bornova Veterinary Control Institute, İzmir, Turkey

Biorisk management system which is effective, sustainable and auditable plays a key role in reducing or eliminating biological risks. Biosafety and biosecurity measures strengthen the system by affecting the probability and / or consequences in preventing the risks caused by biological agents or toxins, including viruses, bacteria, parasites, fungi and prions. Carrying out the system on the basis of Assessment-Mitigation-Performance (AMP) increases the success in reducing biological risks by ensuring that all components are questioned equally. The Biorisk Management Committee included in the Biorisk Management System established in accordance with CWA 15793 (CEN Workshop Agreement - Laboratory biorisk management standard) in the institute carry out its activities according to AMP model. The Committee convenes routinly in every 3 months and when necessary urgently in order to evaluate and analyze the risks and implement the necessary mitigation measures, and plans and policies related to manage of biological risks are determined. The Committee provides necessary trainings on biorisk management and advises laboratories. Each laboratory conducts its own risk assessment, but the final decisions are taken together by the laboratory and the commission.

Performance is one of the three indispensable components of the AMP system and should be conducted to determine whether the system is working correctly. In this study, internal visits were implemented by biorisk committee to strengthen the performance which is routinely carried out through internal and external audits, simulations and records. In this context, biorisk visits were conducted to the Virology, Bacteriology, Parasitology, Pathology and Poultry Diseases diagnostic laboratories in the institution. Information and ideas about risk mitigation were exchanged in the visited laboratories. In addition, laboratory risk assessments were done by another eye. The importance of communication in managing biological risks has been revealed through on-site investigations. Awareness has increased in both the biorisk management committee and the relevant laboratory experts. Considering the results of the visits in terms of institutional needs, the demand for personal protective equipment doubled and the variety of PPE currently used were increased, compared with previous year. It has been observed that small modification in some laboratory layouts in order to mitigation of biological risks.

In a conclusion, the importance of biorisk visits to different laboratories by different experts was revealed in the strengthening of the performance of the biorisk management system. According to the results of biorisk visits, it is understood once again how important risk communication is, and it has been concluded that increasing the number of informed or uninformed visits will be beneficial for biorisk management.

NP5

COVID-19 infection among medical staff: incidence and risk groups - Armenia, 2021

Romella Abovyan[1], Gennady Palozyan[1], Shoghik Avetisyan[1], and Artavazd Vanyan[2]
1- NCDC, MoH of Armenia, Department of Epidemiology of Infectious and Non-Communicable Diseases, Yerevan, Armenia; 2- NCDC, MoH of Armenia, Yerevan, Armenia

Introduction: The COVID-19 pandemic is a severe challenge for medical staff safety. The aim of this study was to evaluate the incidence of COVID-19 infection among medical staff of Medical centers (MCs) in Armenia and identify which medical staff are most at risk.

Methods: The list of staff infected by COVID-19 for the period of 03/2020-04/2021 was collected from all medical institutions of Armenia by the National Center for Disease Control and Prevention (NCDC) of the Ministry of Health (MoH) of Armenia and analyzed using Microsoft Excel and Epi Info software.

Results: NCDC reported 6,323 cases among MC staff: 913 (14%) male and 5,410 (86%) female. The mean age was 48.1 ± 0.2 yr. The majority of cases were detected in the 50-59 and 60-69 age groups. Out of all cases, 3,941 (62%) persons work in the capital MCs, 2,382 (38%) in the regional MCs. There were 1,416 (22%) cases from primary care MCs and 4,907 (78%) from hospitals. Out of 6,323 workers, 30% were doctors, 44% nursing staff, 12% junior nursing staff, and 14% administrative and other staff. Among doctors, mainly physicians, surgeons, pediatricians and family physicians were affected. In 19% of the cases, medical staff were infected outside of the hospital, with an additional 19% of cases where the infection source is unknown.

Conclusion: Out of the >200,000 reported COVID-19 cases in the country, MC staff constituted 3% of the total cases. It is necessary to conduct continuous education of staff and implementation of crucial core components of Infection prevention control (IPC) programs for staff protection. Appropriate use of PPE and adequate hand hygiene should be implemented in all facilities.

NP6

Design and establishment of an Ukrainian in-house diagnostic tool for Detection of *Bacillus anthracis* genetic material by Real-Time PCR

Oleksandr V. Biloivan[1], Borys T. Stegniy[2], Mykhailo V. Gladiy[3], Vasyl V. Vlizlo[4], Anton P. Gerilovych[5], Claudia Popp[6], and Julia Schwarz[6]
1- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Genetics of Microorganisms, Kharkiv, Ukraine; 2- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Director, Kharkiv, Ukraine; 3- National Academy of Agrarian Sciences of Ukraine, Kyiv , Ukraine; 4- National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine; 5- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Veterinary Epizootology, Diagnostics and Quality Control of Genetic Resources, Kharkiv, Ukraine; 6- Bundeswehr Institute of Microbiology, Munich, Germany

Introduction: The challenge of anthrax disease for human and animal health is still acute in many countries of the world. For improving pathogen detection, development of PCR tests for a simple and fast analysis of suspect samples in diagnostical laboratories is paramount. Currently, there is no effective domestic PCR diagnostic tool for the

PCR-detection of *Bacillus anthracis* in Ukraine. Moreover, use of imported analogous is forbiddingly expensive due to high unit costs of reagents and shipping.

Objective: The goal of the work was to design and establish a domestic equivalent of a *B. anthracis* qPCR test.

Materials and Methods: The new test was adapted from previously-developed recombinant positive controls, namely *cloned-pagA* (from plasmid pXO1) and *capC* (from plasmid pXO2) *fragments* and conducted using hydrolysis-probe reagents. Species specificity was validated using DNA samples of various pathogens causing infectious diseases that may be confused with anthrax. Performance of the test was compared to an established PCR assay employing primer oligonucleotides and [rpbes recommended by the OIE for the detection of anthrax plasmid DNA: PA5/8 (*pagA* gene) and 1234/1301 (*capC* gene).

Results: The new diagnostic PCR test detected *B. anthracis* plasmid DNA in various dilutions in samples resulting in threshold (Ct) values of 25.3 to34.7. Conversely, samples not containing *B. anthracis* DNA but DNA of other pathogens were negative. This confirmed the specificity of the new test. In addition, repeatability and reproducibility was proven. The test included differently color-labeled probes allowing multiplex qPCR runs for simultaneous detection of both pXO1 and pXO2 plasmid DNA. This is both time and reagent saving and simplifies overall procedure.

Conclusions: The developed test meets the OIE requirements for specificity, sensitivity and repeatability. This test can be expected to be used for rapid diagnostics of the anthrax pathogen in Ukraine once the formal registration process is completed.

NP7

Study of the diversity of *Leptospira* serovars and molecular epidemiology of Leptospirosis in Georgia

Nazibrola Chitadze[1], Nana Mamuchishvili[1], Janna Pollakova[2], Roena Sukhiashvili[1], Michaela Kreitmeier[2], and Heiner von Buttlar[2]
1- National center for Disease Control and Public Health, Virology, Molecular Biology and Genome Research, Tbilisi, Georgia; 2- Bundeswehr Institute of Microbiology, Munich, Germany

Leptospirosis is a zoonotic disease with worldwide distribution and increasing prevalence caused by spirochetes of the genus *Leptospira*. It is widely distributed throughout the country of Georgia. The highest incidence was reported in 2019 with 11 (409 cases) per 100 000 population, including 9 lethal cases. In 2020, 153 cases of leptospirosis were registered. Incidence was 4.11 (per 100 000 population), including two with lethal outcomes. The main goal of this study is to identify circulating *Leptospira* spp. and evaluation of reservoir-human serotype relationship. Pathogenic leptospira are mainly transmitted by urine of small rodents and therefore during the 2018-2020 period the sampling of rodents was carried out. Blood samples as well as different organs were collected for molecular and bacteriological analysis for the presence of leptospira. According to reported leptospirosis cases in Georgia, sampling sites in the Western parts of the country (Batumi, Kobuleti, and Kutaisi) with different biotopes (forest, bushes and swamps) were chosen.

After the dissection of euthanized rodents, isolation was attempted by inoculating aseptically collected kidney tissue extracts of rodents into 3 test-tubes that contained 4 ml of EMJH medium with inhibitors. The tubes with the inoculated material were incubated at 28° to 30°C and controlled each 7-10 days for the growth of leptospira during 35 to 45 days.

During these field studies 310 rodents in total were caught. Cultural isolation was possible in 14 samples. Leptospira positive cultures were confirmed by quantitative real-time polymerase chain reaction (qPCR) and microscopic agglutination test (MAT). The most prevalent serovars identified by MAT were the groups *Ballum* and *Pomona*.

Our work underlines the importance of rodents as natural reservoir hosts of pathogenic leptospira in the country of Georgia.

This work is part of the German Biosecurity Programme: Establishment of a Western Asian Network for the Improvement of Biosecurity in the Caucasus Region.

NP8

Can NGOs become an important actor in biosecurity education? The German Association for Synthetic Biology"s biosecurity program

Hendrik P.A. Cooper[1], Svenja Vinke[2], Fabian Rohden[3], and Irina Rais[4]
1- GASB - German Association for Synthetic Biology, Steering Committee - Head of Steering, Darmstadt, Germany; 2- Center for Biotechnology (CeBiTec), Bielefeld University, Biology, Bielefeld, Germany; 3- University of Lethbridge and GASB - German Association for Synthetic Biology, Steering

Committee, Lethbridge, Canada; 4- University of Cologne, Biochemistry, Cologne, Germany

The state-of-knowledge on biosecurity among life sciences students is devastating. According to a survey conducted within the synthetic biology community in 2018, 59 % of the participants did not know what dual use in the context of science is and 76 % want a more pronounced education on dual use issues. The unawareness on dual use issues within the life sciences research community is a long-known problem and several national and international organisations reacted by publishing teaching material online, but the vast majority of dual use awareness programs rely on the self-initiative of lecturers to integrate these teaching materials within the universities curricula. An approach that, according to the survey"s data, does not seem to work. The freedom of education makes mandatory biosecurity education not feasible, and most universities act hesitant to incorporate governmental led teaching programs. To solve this dilemma, NGOs can play an important role in developing frameworks for biosecurity education and support universities with little expertise, or insufficient resources, in biosecurity teaching. The German Association for Synthetic Biology (GASB) is currently designing a workshop serious on biosecurity to be held at German universities and offers information, support, and contact persons in case students or researchers have concerns about their research projects. Join us at our poster to discuss how NGOs can best support biosecurity education and to learn more how the GASB takes up this task to support synthetic biology students becoming responsible researchers!

NP9

Plague epizooty situation in Mongolia and risk of human disease

Tserennorov T. Damdindorj[1], Baigalmaa B. Molotov[2], Gankhuyag K. Tsedev[2], Otgonbayar O. Damdin[2], and Tsogbadrakh T. Nyamdorj[1]
1- National center for zoonotic diseases, Administration, Ulaanbaatar, Mongolia; 2- National center for zoonotic diseases, Epidemiology, Ulaanbaatar, Mongolia

Background: Mongolian plague natural foci occurs most percent of Central Asian foci. In Mongolia, 137 soums of 17 provinces have plague natural foci and human cases have particularly associating with marmot hunting in there. Although, since 2000, human cases of plague have been decreasing gradually, reported 1-3 cases per year, natural foci is still with a high active. Thus, our study aimed to estimate plague epizooty situation of Mongolia between 2000 and 2020.

Materials and method: Data from the plague surveillance conducted in 13 aimags in 2000-2020 were collected from the report and retrospectivity analyzed. Used MS Excel, Arc View 3.2 and QGIS programs.

Results: In 2000-2020, natural foci surveillances were conducted in 13 aimags and an epizooty had been identified in the total of 55 soums (2 in Arkhangai aimag, 7 in Bayan-Ulgii aimag, 4 in Bayankhongor aimag, 8 in Gobi-Altai aimag, 9 in Zavkhan aimag, 4 in Uvurkhangai aimag, 1 in Tuv aimag, 2 in Uvs aimag. , 10 in Khovd aimag, 3 in Khuvsgul aimag, 3 in Khentii aimag, 1 in Umnugovi aimag, 1 in Dornogovi aimag).

During the study, 479 Y. pestis strains were detected from 11 species of rodents, rabbits and 3 types of parasites, of which 84.8% were marmots, their carcasses and fleas.

Depending on the condition of the foci, 46-128 cultures were detected in more than 5 soums of the aimags Khovd (10), Gobi-Altai (8), Zavkhan (9), Bayan-Ulgii (7) and epizootics were identified. 102 cultures were detected in 3 soums of Khuvsgul aimag due to the active monitoring team conducted in 2000 in Tsetserleg soum of Khuvsgul aimag.

The highest percentage of total plague strains (31.1%) was detected in Tsetserleg soum of Khuvsgul aimag and Ulaankhus soum of Bayan-Ulgii aimag. The natural foci of marmot epidemics are geographically identified in forest-steppe, mountain steppe, steppe and mountainous areas, indicating the wide range of plague epizootic.

The detection period of plague strains begins in May and lasts until October, but increases steadily until September, peaking in August and September, and declining in October.

In 2000-2020, Nogoonnuur, Ulaankhus, Tsagaannuur of Bayan-Ulgii aimag, Must, Uench, Bulgan, Mankhan of Khovd aimag, Tsetserleg of Khuvsgul aimag, Tsagaan-Uul, Erdenekhairkhan, Yaruu, Telmen of Zavkhan aimag, Tugrug, Tsogt and Erdene soums of Gobi-Altai aimag studies have shown that plague epizootics are highly active in natural plague foci.

In the 2000-2020, 61 plague human cases were registered in aimags and soums with epizootics, except for 2005, 2012, 2016, and 2018, and the mortality rate was 32.8% (20).

More than 70% patients were infected through direct contact with marmot such hunting, skinned and butchering, more than 20% by flea bites and the remaining percentage from infected people and by ingestion of raw marmot organs.

Conclusion:

1. Detection of plague epizootics is likely to depend on the frequency and duration of surveillance.
2. In last 20 years, according to isolated strains in 55 soums of 13 aimags" natural foci that at average 23 strains were detected in 55 soums yearly, shows that the natural foci is highly active.
3. As marmots are the primary source of plague human cases, it is important that people do not engage in risky behaviors.
4. Due to the high risk of plague human cases, there is a need to improve the behavior and knowledge of the people, such as not to hunt illegally and not to use raw marmot organs, and it is important for professional organizations to cooperate.

NP10

Prevalence of lumpy skin disease virus(LSD), analysis of epidemiological data in Georgia

Larisa Derkach
Kutaisi Zonal Diagnostic Laboratory, LEPL State Laboratory of Agriculture of Georgia, Kutaisi, Georgia

Introduction: Lumpy skin disease virus (LSDV), a member of the capripoxvirus genus of the Poxviridae, is a viral disease in cattle. This disease is an important transboundary cattle disease and has recently spread from Africa to Europe (South to Northeast). The disease causes considerable devastating economic losses mainly due to permanent hide damage, milk production, abortion and infertility, and emaciation and disruption in the trade of cattle and their products. Capripoxviruses are agents for agroterrorism and are listed as a disease since they cause serious economic losses. Lumpy skin disease virus has been declared as a disease subject to registration by the International Animal Health Organization due to veterinary and economic significance. Lumpy skin disease is a recent disease for Georgia. In July 2015, this disease was discovered on the border of Georgia and Azerbaijan. In 2016, SLA diagnosed 22 positive cases of lumpy skin disease by PCR research, 4 positive cases in 2017, and 7 positive cases in 2018.

Method: Polymerase chain reaction (PCR) is the most economic and quickest method for the detection of LSDV. Skin nodules and scabs, saliva, nasal secretions, and blood are suitable samples for PCR detection of LSDV. Specific forward and reverse capripox primers were used: Forward Primer-5'-TCCGAGTTTCCTTACTAT-3'; Reverse Primer 5'-TATGGTACCTAAATTATATACGTAAATAAC-3'; Probe 5'-6FAM-CAATGGGTAAAAGATTTCTA-MGBNFQ-3'. PCR master mix was prepared using Taq polymerase,10x PCR buffer, 50 mM MgCl2, and 10 mM dNTPs according to the protocol. Real-time PCR is a rapid, sensitive, and specific method for confirmation of capripoxviruses including LSD.

Conclusion: To prevent further spread of the virus, the National Food Agency vaccinated the cattle in the risk regions. Given the expected risks, 46,908 cattle were vaccinated in 2014; in 2015-181 08 cattle; in 2016-234 978 cattle; in 2017-291 527 cattle; in 2018-738 180 cattle; in 2018-476 918 cattle. The effectiveness of the vaccination campaign is confirmed by the fact that in 2019 there were no recorded cases of LSDV.

NP11

Response to COVID Pandemic in dental care settings in Khartoum state, Sudan: Interventions, challenges and the way forward

Maisa M. Elfadul[1], Arwa O. Saeed[1], Samar A. Abushook[2], Yousef Khader[3], and Munitisir A. Alfadul[4]
1- University of Medical Sciences and Technology, Public and Tropical Health Programmes, Khartoum, Sudan; 2- Napata College, Conservative Dentistry, Khartoum, Sudan; 3- Jordan University of Science and Technology , Public Health, Community Medicine, Irbid, Jordan; 4- Almontasir Centre for Implantation, Khartoum, Sudan

Background: The new pandemic of COVID-19 disease has created extreme challenges for both dental and medical practices at all sectors whether public, private, or educational. There is dearth of knowledge about the experience of low middle-income countries and their approaches to sustain dental services during COVID-19 pandemic. Although many Interventions have been suggested to ensure the continuum of dental services, yet limited evidence is currently available on the impact of such interventions on improving service delivery.

Objectives: The aim of this study was to document the experience of Sudan response to COVID-19 within the dental care setting through highlighting health care interventions along with challenges associated with implementation.

Methodology: The study followed the operational research methodology where all interventions in dental care have been identified then bottle necks and practical solutions have been suggested. The study

employed the phenomenology approach and data collected through key informants" interviews followed by document review for the interventions report.

Results: The study documented all the six interventions implemented by the Oral Health Department at the Khartoum State Ministry of Health during wave one COVID-19 Pandemic. The interventions included Provision of infection control supplies for dental hospitals, Closure of private clinics and dental centers, Establishing a protocol for emergency hospitals, Facilitating delivery of emergency dental services, Online dental consultations center, and Reviewing and updating clinical protocols for reopening of private sectors. The challenges encountered during implementation of these interventions were reported following the health system building blocks framework.

Conclusion: Dental care setting in Khartoum have managed to provide affordable sustainable emergency services during COVID-19 pandemic, but still many challenges related to governance, human resources for health and logistics were encountered which undermine safe service delivery.

NP12
Human Brucellosis in Tunisia

Asma Ferjani[1], Hellen Buijze[2], Amel Rehaiem[1], Jacob Gatz[3], Nicole Hanschmann[3], Claudia Sievers[3], Meriem Zribi[4], Holger Scholz[3], and Ilhem B. Ben Boubaker[1]
1- Charles Nicolle Hospital, Tunis, Tunisia; 2- Highly Pathogenic Microorganisms, Centre for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany; 3- Highly Pathogenic Microorganisms, Centre for Biological Threats and Special Pathogens Robert Koch Institute, Berlin, Germany; 4- La Rabta Hospital, Micobiological department, Tunis, Tunisia

Brucellosis is a serious zoonotic disease and is endemic in the Mediterranean. In Tunisia, diagnosis of human Brucellosis is largely dependent on serological testing and bacterial culture with no insight into the causative Brucella species or biovar. Furthermore, while MLVA analysis and more recently WGS-based typing technologies have given insight into species diversity and strains circulating in the Mediterranean, missing WGS data for North-African strains present gaps in the Brucella population structure.

In the framework of the German Biosecurity Programme the Charles Nicolle Hospital of Tunis in collaboration with the Robert Koch Institute in Berlin has established real-time PCR and conventional PCR assays to allow molecular diagnosis of *Brucella* spp. as well as differentiation into the clinically most important species. Retrospective PCR analysis of 45 isolates from clinically diagnosed Brucellosis cases between 2015 and 2020 confirmed the diagnosis of 43 samples, which were all identified as *B. melitensis*. In addition, all 36 cultivable isolates were subjected to WGS and compared to a database of almost 1000 Brucella genomes in a cgMLST scheme based on 2678 genes. All Tunisian strains belonged to B. *melitensis* bv3 and mostly grouped into two new major clusters with close genetic relationships to strains from neighboring countries. Markedly, strains from a recent Brucellosis outbreak in Austria with yet unidentified infection source grouped closely together with strains from Tunisia, suggesting that North-Africa might represent the geographical origin. The latter finding highlights the importance of filling geographic WGS data gaps to allow molecular-epidemiological investigations of natural outbreaks at a global scale and to determine potential infection sources

NP13
Complex work to prevent the spread of FMD in Imereti region of Georgia

Manana M.G. Gabrichidze
Kutaisi Zonal Diagnostic Laboratory, LEPL State Laboratory of Agriculture, Specialist of Department of Sample Receiving and Anatomical Pathology. Molecular Laboratory Specialist, Kutaisi, Georgia

Introduction: In everyday life, we learn about human health problems through their interactions with animals and the environment. Severe zoonotic diseases continue to have serious negative impacts on health, livelihoods, and the economy. Today's global pandemic has further clarified these issues. Among those severe diseases is Foot and mouth disease (FMDV), an acute and rapidly spreading zoonotic disease of cloven-hoofed animals. The source of infection of animals and humans is infected cattle, their products, and excrement containing the causative agent of the infection. The outbreak affected many countries, endangered human health, had a devastating effect on the country's economic losses and international trade, and was informally called the "economically devastating disease of livestock".

Goals and objectives: Foot and mouth disease continue to rank first in the category of most serious infections. Currently, there are seven types of viruses: A, O, C, SAT-1, SAT-2, SAT-3, and ASIA-1, each type has its parameters and their number is constantly increasing. For prevention, complex work is important: laboratory data, epidemiological indicators of foot and mouth disease in the region,

vaccination against foot and mouth disease of all assimilated animals. Kutaisi Zonal Diagnostic Laboratory have conducted ELISA diagnostic analysis of FMD Non-Structural Proteins (NSP) of received samples in the framework of sero-monitoring, as well as from private sector. In 2017, the laboratory tested 322 blood serum samples from cattle and breeding cattle in the region with 11 positive results. In 2018, 294 samples with 8 positive results; in 2019, 5887 samples with 149 positive results; in 2020, 4205 samples with 52 positive results. After the initial diagnosis, the Kutaisi Zonal Laboratory sent positive samples to Tbilisi laboratory for serotyping.

Conclusion: The risk of spreading zoonotic diseases is high. The outbreak covers large areas beyond state borders and causes significant, sometimes catastrophic, socio-economic damage. The benefits of using One Health's multidisciplinary approach to address health risks are recognized in many states. The Kutaisi Zonal Diagnostic Laboratory is actively involved with the US Defense Threat Reduction Agency (DTRA) in the framework of the Joint Biological Cooperation, in the One Health Program and is fully prepared for timely intervention in the event of an epidemic to reduce the expected threat.

NP14

European Mobile Laboratory Deployments for Diagnostic Support in the COVID-19 Pandemic

Martin Gabriel[1], Sophie Duraffour[2], Stephan Guenther[2], Alexander Schlaphof[1], Martin Richter[3], Janine Michel[4], Jessica Kehrer[5], Lien de Caluwé[6], Beate Becker-Ziaja[7], and Emily Nelson[2]
1- Bernhard-Nocht-Institute for Tropical Medicine, Central Laboratory Diagnostics, Hamburg, Germany; 2- Bernhard-Nocht-Institute for Tropical Medicine, Department for Virology, Hamburg, Germany; 3- German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany; 4- Robert-Koch-Institute, Diagnostics, Berlin, Germany; 5- University Hospital Heidelberg, Heidelberg, Germany; 6- Institute for Tropical Medicine, Antwerp, Belgium; 7- Robert-Koch-Institute, Berlin, Germany

The European Mobile Laboratory (EMLab) was established in 2011. Since then, several field deployable lab units were set-up and experts were trained for worldwide deployment in outbreaks. EMLab missions are supported by partners from Europe and Africa. Lab units were deployed on several occasions: Ebola virus outbreak in West-Africa 2014 to 2016, yellow fever outbreak in the Democratic-Republic of the Congo in 2016, and Marburg virus outbreak in Uganda 2017. EMLab is a partner of the WHO Global Outbreak Alert and Response Network (GOARN) and registered with the European Medical Corps of the European Commission (DG-ECHO). It is usually deployed following request for assistance from WHO-GOARN and missions are supported by the Global Health Protection Programme (GHPP) of the German Ministry of Health, WHO, and DG-ECHO.

Early in 2020, the COVID-19 pandemic started. In March 2020 several COVID-19 clusters occurred in Germany. One of these hotspots was in Weiden in der Oberpfalz in Bavaria. The regions of Neustadt a.d. Waldnaab, the district of Tirschenreuth and the city of Weiden suffered from a surge of COVID-19 cases overwhelming diagnostic capacity. A state of disaster was proclaimed and an EMLab unit was requested to support with SARS-CoV-2 molecular diagnostics at the general hospital in Weiden. From 24th of March to 28th of April 2020 one EMLab unit was deployed with in total eight team members. The transport was supported by the German Agency for Technical Relief (THW). EMLab performed SARS-CoV-2 testing with a short turn-around time, trained local staff, and assisted the hospital in establishing its own COVID-19 diagnostics unit within the central lab facilities.

On September 8th 2020, a fire burned down the Moria refugee camp on the Greek island of Lesvos. A new site has been set up in the area of Kara Tepe including a new health centre. Following the request for assistance from the Greek Ministry of Health, one EMLab unit was deployed to Kara Tepe camp with the support of WHO-GOARN to assist in the medical management of COVID-19 cases and infection control by providing molecular SARS-CoV-2 diagnostics.

Between 4th October 2020 and 28th February 2021 six teams with in total over 30 laboratory experts were deployed. The lab unit performed SARS-CoV-2 RT-PCR testing for beneficiaries and staff, as well as clinical lab analysis for patient management. EMLab also trained local staff and handed-over lab equipment for sustained operation of a lab in the camp.

NP15

Incidence of COVID-19 in L"viv, Ukraine, 2020-2021

N Ivanchenko, O Brezetska, and O Karkishchenko
State Institution L'viv Oblast Laboratory Center of the Ministry of Health of Ukraine, Kyiv, Ukraine

An outbreak caused by a new viral pathogen is accompanied by uncertainty about clinical signs, and risk factors for infection. This study examined

all confirmed cases of COVID-19 in L"viv oblast to identify key epidemiological characteristic and to analyze the distribution by age and gender. We reviewed epidemiological records from the case database EIDSS.

From the first confirmed case in March of 2020 through 3/20/21, L"viv Oblast has accounted for 6% (92,420) of all confirmed cases in Ukraine (1.54M). Incidence of COVID-19 in L"viv oblast was 3,681 cases per 100 thousand. The incidence rates in the Peremyshliany (4131.9 cases per 100 thousand), Pustomytiv (6989 cases per 100 thousand), Mykolaiv (4639 cases per 100 thousand population) raions and L"viv (5896,3 cases per 100 thousand) are above the average rate in the Oblast.

In L"viv oblast, 2,474 people with confirmed COVID-19 died from the first case in March of 2020 up until 3/20/2021. The mortality rate was 97.7 per 100 thousand and the case fatality rate was 2,7%. In Ukraine, these indicators were 78.2 per 100 thousand population and 1,9%, respectively. Fifty-nine percent of all confirmed cases were females and 74.5% of all confirmed cases were of people from 30-69 years old.

Among confirmed cases in L"viv oblast. Fifty-five percent of COVID-19 confirmed deaths were males and 92.5% of COVID-19 confirmed deaths were from people aged 50-79 years old.

Our data shows that in L"viv Oblast the most confirmed cases are registered among women aged 30-69 years. The case fatality rate exceeded the average Ukrainian indicators. The lack of information on the number of tests is a limitation in our study.

NP16

Development of PCR and ELISA based diagnostic tools for brucellosis surveillance study in Ukraine

Mykola Kalashnyk[1], Claudia Popp[2], Julia Schwarz[2], Anton Gerilovych[1], Vitaliy Bolotin[1], and Heiner von Buttlar[2]
1- National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine; 2- Bundeswehr Institute of Microbiology, Munich, Germany

Brucellosis remains an important zoonotic infectious disease causing economic losses due to the reproductive failure in animals all over the word. Ukraine is currently free of bovine and swine brucellosis. However, there are many risks of its recurrence due to the endemic situation in neighboring countries and by spreading among wild animals. Therefore, there is the need for effective diagnostic tools such as ELISA and PCR to perform brucellosis surveillance studies. In the framework of the "Ukrainian-German Biosecurity Initiative for Risk Management of Zoonosis close to the European Border" a qPCR targeting the gene IS711 for brucella detection and an indirect ELISA for the detection of Brucella-specific antibodies based on recombinant antigens will be developed.

The gene IS711 is highly conserved among all Brucella spp. strains. Therefore, it was chosen as target sequence for a diagnostic PCR. For the development of the qPCR we started with the preparation of the positive control using the vector pTZ57R and the primer pair IS711_S & IS711_R (Tomaso H. et al., 2010). First results on validation are going to be shown.

The lipopolysaccharide (LPS) in the cell wall of Brucella is considered the most important antigen for the immune response during infection. To overcome the cross-reactive nature of LPS with other Gram negative bacteria, the outer membrane protein Omp26 of Brucella abortus was chosen as the recombinant antigen for the development of an indirect ELISA. Brucella outer membrane proteins (OMPs) have been evaluated as a non-LPS group of immunogens and have received major attention for their use in diagnostic systems for various zoonotic diseases, including brucellosis. For the serological assay, the protein is engineered to be overexpressed in E. coli BL21 with a 6xHis-tag, by cloning into pET28a(+) vector and single step purification is done under native conditions. To ensure intracellular expression of the protein, the sequence coding for the signal peptide was removed. The purified soluble recombinant protein will be analyzed for its immunogenicity by Western blot and in ELISA.

NP17

The importance of implementing training programs in improving Laboratory biosafety and Biosecurity in Sudan

Elameen A. Mohammed, Ceza M. Obeid, Huyam O. Mohamed, and Sanaa M. Babikir
National public health laboratory , Biorisk department, Khartoum, Sudan

Sudan occupies a great area in the African continent. As it shares borders with seven countries, it has a wide diversity in environment and climate.

Sudan is an area at great risk of communicable diseases outbreaks, in both human and animal population. Moreover the state economy basically depends on livestock and animal resources which are considered as natural reservoir for many pathogens.

Sudan is a key partner in German biosecurity program for Excellence in Biological and Health security. This program was launched within the engagement of the German Federal Foreign Office within its commitment in the "Global Partnership against the Spread of Weapons of Mass Destruction" in 2013.

The German-Sudanese partnership aims to:

- Improve mitigation of biological risks
- Enhance Sudanese public health capacities
- Fostering international scientific exchange and quality scientific personnel
- Contribute to the fight against dangerous diseases
- Support safe and secure conditions for working within dangerous pathogens and toxins.

The Germany Sudanese partnerships conducted a lot of training activities in biosafety and biosecurity in collaboration with Robert Koch Institute in order to build the capacity of Sudanese working in public health area from different sectors which are involved in health services delivering or involved in research activities related to human and animal health. These include health, veterinary, higher education and research sectors. The activities involved series of workshops in training of trainers aiming at establishment of sustainable implementation of biosafety and biosecurity education and training in Sudan. As a result of these activities a core group of 14 trainers representing 6 different stakeholders and partners has been built. This group is now capable of conducting Bio-safety and bio-risk training independently.

In the second phase of this partnership 18 focal point from the 18 different states of Sudan are targeted as trainers, this time the training will be conducted jointly by Sudanese and German trainers.

This presentation will explain the importance of training the trainers, overview of the 8 modules which constitute the main subjects of the training and the experience that gained in implementing such kind of this training in Sudan specially in the area of biosafety and biosecurity that linked with facility and safety such as designing ,good microbiological practice and procedures and biosecurity during outbreaks .

NP18

Registered cases of the use of modified types of the causative agent of the Crimean-Congo hemorrhagic fever in Kazakhstan

Gulnar Omasheva[1], Alim Aikimbayev[2], and Zangar Turliyev[3]

1- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Reference laboratory for the control of highly dangerous infections, Almaty, Kazakhstan; 2- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Scientific consultant, Almaty, Kazakhstan; 3- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Department of Risk Assessment and Epidemiology of Infectious Diseases, Almaty, Kazakhstan

Crimean-Congo hemorrhagic fever is an arbovirus transmissible natural focal infectious disease caused by the Crimean-Congo hemorrhagic fever virus, transmitted mainly through tick bites.

CCHF was first reported in 1948 (detected in South Kazakhstan oblast). Despite the large-scale disinfection campaigns in natural foci in the southern part of Kazakhstan, the sporadic incidence in humans is still there, and cases of CCHF in people, and lethal cases are being registered every year.

However, the last years have demonstrated an interesting trend when people often get infected with a disease clinically identical to CCHF, but the laboratory testing does not confirm the agent.

CDC epidemiologists raised the fact in one of the meetings saying that in the studies conducted with participation of people who were diagnosed with potential CCHF case, the laboratory tests only confirmed 30% of those cases. It is unknown why the remaining 70% have not been confirmed.

Epidemiologists from endemic areas also confirmed facts where people had symptoms very similar to CCHF symptoms with hemorrhagic syndrome, clinical progression and incubation period shorter than with regular CCHF.

Epizootic and epidemiological studies of CCHF situation from 2017-2019 showed that 140 patients across the country had potential CCHF cases and were admitted to infectious diseases hospitals for treatment. But only 18 patients have been confirmed to have positive CCHF cases verified by the laboratory tests. The remaining 122 patients were diagnosed with different infection (Varicose veins - 26, rickettsia - 2, other - 94).

In Zhambyl oblast, out of 39 cases of potential CCHF only 6 cases (15,3%) and in Turkestan oblast, 2 out of 76 cases (2,6%) were confirmed CCHF cases. "The situation requires a diagnostics approach different from applied towards other infections", - concluded the Center"s epidemiologists.

Experts from EDP reference laboratory conducted their own research: in 2018, they found out that 5 out of 74 samples collected from patients with

potential CCHF in Zhambyl oblast, except for 8 cases confirmed both clinically and by laboratory testing, also had RNA of CCHF agent. However, no IgM or IgG antibodies against CCHF have been detected in those patients. A mutation might have happened in the genes of this especially dangerous virus which makes the laboratory diagnostics more difficult. This situation requires scientific research to identify true agents of the diseases similar to CCHF.

NP19
Surveillance over anthrax in Kazakhstan

Gulnar Omasheva[1], Alim Aikimbayev[2], and Gregor Grass[3]
1- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Reference laboratory for the control of highly dangerous infections, Almaty, Kazakhstan; 2- Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring , Scientific consultant, Almaty, Kazakhstan; 3- Institute for Microbiology of the German Armed Forces, Dept. of Bacteriology and Toxinology, Munich, Germany

Anthrax is an extremely dangerous infectious disease for humans, associated with specific edema and necrotic lesions on the skin and internal organs and a severe intoxication.

Economic development of Kazakhstan implicates an expanded growth of the livestock sector associated with development of grassland farming that may cover natural foci of especially dangerous infectious pathogens. Infections with anthrax were caused though contacts with infected soil, and isolation of the agent from soil samples collected from animal burial sites evidenced the fact that the potential danger of infection coming from soil foci of anthrax lasts for many decades. In Kazakhstan, livestock and wild animals can serve a source of infection. The anthrax agent, Bacillus anthracis, is transmitted to humans through contact with infected animals, their carcasses, infected products of animal origin, when consuming not properly heat-treated infected meat and meat products, as well as when performing any ground works and thus, contact with a contaminated soil.

Annually, we report cases of anthrax in people, where foci are confirmed, by lab testing, including bacteriological isolation of strains. In 2018, anthrax isolates were obtained from a patient in the East-Kazakhstan oblast. Bacteriological tests showed contamination of environmental samples at the anthrax foci at the level of 0.2%. A total of 20 B. anthracis isolates were recovered in the East-Kazakhstan oblast: from meat products (7), from animals (13; 10 from meat samples and 3 isolates from lesions).

In 2019, 5 isolates of anthrax have been recovered from patients including 2 isolates obtained in Turkistan, and Akmola, Zhambyl oblast and Shymkent obtaining one isolate each. Contamination of environmental samples with anthrax at the natural foci sites reached 0,3%, 33 B. anthracis isolates have been obtained overall including four isolates from meat products (1 in Akmola oblast and 3 in Turkistan), from infected feed – 1 isolate in Akmola oblast, from soil – 1 in Akmola oblast, 2 in Turkistan oblast, from infected hides and wool of livestock animals – 1 in Akmola oblast, 24 anthrax isolates obtained from livestock animals (Akmola-9, Turkistan-7, Pavlodar – 6, Shymkent - 1, Nur-Sultan - 1).

In 2020, there were 5 B. anthracis isolates confirmed by the reference laboratory, 4 isolates obtained from patients in Zhambyl oblast (1) and in Shymkent (3).

Therefore, isolation of the agent from different samples may improve the understanding of its epidemiology and phylogenetics, This is why it is relevant for Kazakhstan to conduct molecular-genetic testing of strains for surveillance of this notorious pathogen.

NP20
Study of leptospirosis infection transmission risks in the Kharkiv region of Ukraine

Nataliia Rudova[1], Oleksandr Zinenko[2], Claudia Popp[3], Vitaliy Bolotin[1], Heiner von Buttlar[3], Boris Stegniy[1], Anton Gerilovych[1], Oleksii Solodiankin[1], and Julia Schwarz[3]
1- NSC IECVM, Kharkiv, Ukraine; 2- V. N. Karazin KhNU, Kharkiv, Ukraine; 3- Bundeswehr Institute of Microbiology (IMB), Munich, Germany

Introduction: Rodents and lagomorphs (rabbits, hares, voles, rats, shrews, etc.) and some species of synanthropic birds (sparrows, pigeons) are one of the main factors in the spread of zoonotic diseases, including leptospirosis. The results of a survey conducted among the military personnel show an increase in the number of mice at the places of soldiers" deployment. These are favorable conditions for the formation of long-term natural zoonotic infections foci, such as leptospirosis, which need to be monitored. Starting from 2021, we began to conduct such research in the Kharkiv region of Ukraine.

Methods: For this studies, we collect kidney and lung specimen from rodents. For the detection of pathogenic Leptospira we are going to perform

qPCR analysis using the gene LipL32 as target. As one of the main differential diagnosis for leptospirosis is hantavirus infection, that is submitted by rodents as well, we plan to use the DobV M6 / M8 primers for conventional PCR to detect hantavirus RNA.

Results: We validated real-time PCR with LipL32 primers and probe on Leptospira panels. We got a positive result for all DNA samples of Leptospira. Furthermore, we prepared positive controls for the Leptospira and Hanta specific PCRs. These methods will be used to monitor the prevalence of Leptospira and hantavirus in rodents captured in Kharkiv oblast of Ukraine. So, scientists of NSC "IECVM" received diagnostic tools for detection of leptospirosis and hantavirus infection on the territory of Ukraine, within the framework of Ukrainian-German Biosecurity Initiative for Risk Management of Zoonosis close to the European Border.

NP21

German Biosecurity Programme in Georgia: To Support Trainings and Workshops in Partner Countries

Roena Sukhiashvili[1], Jana Pollakova[2], Amiran Gamkrelidze[1], Michaela Kreitmeier[2], Heiner von Buttlar[2], and Paata Imnadze[1]
1- National Center for Disease Control and Public Health (NCDC), Tbilisi, Georgia; 2- Bundeswehr Institute of Microbiology (IMB), Munich, Germany

A German-Georgian collaboration between the National Center for Disease Control and Public Health (NCDC) and the Bundeswehr Institute of Microbiology (IMB) has been conducted since 2013. The focus of the project lies on minimising the risks associated with biological substances and pathogens and strengthening public health through awareness raising, networking, capacity development, detection and diagnostics, surveillance and biosafety and biosecurity. The goal of the project is achieved through practical workshops, field studies and trainings conducted by PhD students and NCDC personnel trained in the first and second project phase. The ongoing COVID-19 pandemic affected also the start of the third phase of the project in 2020. However, Georgian partners have benefited from the knowledge gained so far and were able to react quickly to the changing situation in the healthcare sector by implementing state-of-the-art methods necessary for the detection of SARS-CoV-2. Despite the situation, number of activities took place last year. An onsite field study with the aim of the identification of the prevailing serovars of *Leptospira* in the country was performed. Seminar on Leptospiroses with the focus on the epidemiological situation in Georgia and diagnostic approaches was held in Adjara region. Workshop on foundation and setting up molecular diagnostic laboratory was conducted in the Local Sentinel Station in Kakheti region.

Molecular-epidemiological study of SARS-CoV-2 with the focus on the genomic structure and genomic differences of the virus over time has been initiated.

Long-standing contacts established within the project between Ministry of Internal Affairs of Georgia, NCDC, National Food Agency in Georgia and IMB will be used in the future for further development of capacities and countermeasures against biological threats. Theoretical as well as practical trainings to strengthen the readiness against bio-terrorism of first responders are planned. Further more workshop on dual use research of concern (DURC) conducted by project participants from Lugar center will be held at the Tbilisi Medical University.

In the framework of the Train the Trainer model, a former PhD candidate of the project will organize and conduct workshops held in five regional laboratories of NCDC with the focus on international standards of biosafety and biosecurity and responsible use of biological materials.

NP22

The Role of the Detection and Diagnostic Components of the German Biosecurity Programme in preparing Partner Institutes for a Global Pandemic

Rebecca A. Surtees[1], Hellen Buijze[2], Jacob Gatz[2], Nicole Hanschmann[2], Natalie Hofmann[1], Eva Krause[1], Olena Kysil[3], Janine Michel[1], Andreas Puyskens[1], Claudia Sievers[2], Holger C. Scholz[2], and Andreas Nitsche[1]
1- Robert Koch Institute, Centre for Biological Threats and Special Pathogens - Highly Pathogenic Viruses, Berlin, Germany; 2- Robert Koch Institute, Centre for Biological Threats and Special Pathogens - Highly Pathogenic Microorganisms, Berlin, Germany; 3- Robert Koch Institute, Centre for International Health Protection – Public Health Laboratory Support, Berlin, Germany

The overarching goal of the German Biosecurity Programme is to aid partner countries in tackling biological threats to prevent the misuse of biological pathogens and toxins. This goal is achieved through strengthening the health capacities of partner countries in Africa, Central Asia and Eastern Europe in six different areas: awareness raising, networking,

surveillance, biosafety and biosecurity as well as detection and diagnostics.

At the Robert Koch Institute (RKI) the detection and diagnostics component can be broadly divided into bacterial and viral detection and diagnostics teams, who work together with six partner institutes in Sudan, Tunisia and Morocco to improve their capacity to detect and diagnose highly pathogenic bacteria and viruses. Examples of these include (but are not limited to) *Brucella* species, *Vibrio cholerae*, *Bacillus anthracis*, MERS coronavirus, Ebola virus, Crimean-Congo hemorrhagic fever virus and Rift valley fever virus.

The capacity building at our partner institutes is carried out in several ways including: training of laboratory staff, establishing, sharing or procuring laboratory assays for the molecular and serological detection of highly pathogenic bacteria and viruses, the procurement of laboratory reagents and equipment, as well as the support of research projects investigating highly pathogenic viruses and bacteria in partner countries. The training of laboratory staff at partner institutions and at the RKI includes comprehensive theoretical and practical training in the detection and diagnostics of highly pathogenic bacteria and viruses, with a focus on their safe handling and inactivation. We also provide partner institutes with External Quality Assessments, in order to gauge/confirm their ability to correctly diagnose these highly pathogenic viruses and bacteria in case of an outbreak.

These activities help to strengthen sustainable safe laboratory practices and create a baseline level of preparedness in our partner institutes in the event of infectious disease outbreaks, including for the on-going SARS-CoV-2 pandemic. In addition, the already established lines of communication through the German Biosecurity Programme enable the continuing support of our partner countries during the SARS CoV-2 pandemic. This poster presents the main actors and gives an overview of milestone activities that the RKI bacterial and viral detection and diagnostic teams have achieved during the course of the German Biosecurity Programme.

NP23

The effect of disturbed gastrointestinal colonization resistance on the incidence and course of the coronavirus infection in the south of Kyrgyzstan.

Rakhmanbek Toichuev
Institute of Medical Problems , Kyrgyz National Academy of Sciences, Osh, Kyrgyzstan

The coronavirus infection that emerged at the end of 2019 in the Chinese city of Wuhan caused a stir in the world community. Even the developed countries of the world community could not withstand the onslaught of this infection. At the same time, 7-8 months passed before the first vaccine was received and full vaccination of the world's population is expected to be completed at the end of 2021, i.e. it takes more than 2 years. Therefore, the scientific community will have to look for ways to save humanity from infection before receiving vaccines and carrying out a complete vaccination.

At the same time, not all "contacted" get sick with coronavirus, moreover, 70-80% of those who get sick have a mild disease. Our study showed that in patients living in uranium biogeochemical zones who fell ill with brucellosis and typhoid fever (TF), a sharp violation of the colonization resistance of microflora in the gastrointestinal tract was revealed, and in a group from the same families who did not get sick colonization resistance of microflora is not disturbed by brucellosis and TF. It is widely known that eubiotics, participating in almost all metabolic processes of digestion, are simultaneously natural antagonists of microbes and viruses, they are directly involved in the formation of secretory immunoglobulins. Based on these, in order to establish the role of the impaired colonization resistance of the intestinal tract on the development of coronavirus, we studied the state of the intestinal biocenosis.

Materials and methods: The study was carried out among those who came to our lab with ARVI, discomfort, intestinal manifestations, 5 patients with subsequent PCR with confirmed diagnosis of coronavirus (group I), group II included 24 contacted people who did not get coronavirus and group III included 22 people who had coronavirus who have contacted us with "residual" manifestations of the disease.

Although the material is not large, it can be assumed that the development of coronavirus infection can be promoted by the disturbed colonization resistance of the intestinal biocenosis, with a decrease in the content of eubiotics and with a sharp increase in the number of low-fermenting E. coli. Therefore, for the prevention of coronavirus, it is necessary to correct the colonization resistance of the intestinal biocenosis, preferably with eubiotics isolated from local strains. But this issue requires a more detailed study based on a large amount of material.

NP24

Establishing Mobile Laboratory Units in Uzbekistan: Conceptions and missions

Gerald Zikeli[1], Gulnora Abdukhalilova[2], Shavkatjon Umurzakov[3], Maximilian Pankla[1],

Malokhatkhon Tashpulatova[2], Mirzarakhim Baynazarov[3], and Kilian Stoecker[1]
1- Bundeswehr Institute of Microbiology, Munich, Germany; 2- Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases, Tashkent, Uzbekistan; 3- Research Institute of Virology, Tashkent, Uzbekistan

Epidemic outbreaks of infectious diseases pose a significant threat to the public health services in many countries, as witnessed in the current global COVID-19 pandemic. Particularly in rural and remote areas, infrastructure and diagnostic capacities are often limited.

In the framework of the European Union Chemical Biological Radiological and Nuclear Risk Mitigation Centres of Excellence (EU CBRN CoE) initiative Project 53, which is implemented by the International Science and Technology Center (ISTC), the Bundeswehr Institute of Microbiology (IMB) has delivered two rapidly deployable diagnostic laboratories (Mobile Lab) to the Ministry of Health of the Republic of Uzbekistan in January 2021.

The Mobile Lab enables the performance of high quality molecular and serology diagnostics of pathogens up to biosafety level 4. It is rapidly deployable to sites of outbreak and eliminates the requirement to transport large quantities of samples for analysis. Thus, it increases Uzbekistan"s capacities in the area of biosafety and biosecurity as well as the national capabilities to prevent prepare for and respond to biological risks.

The conception of the project is based on IMB"s experience during the Ebola epidemic 2014/15 and previous international Mobile Lab projects. With the provision of the Mobile Labs under EU CBRN CoE Project 53, scientists from two leading Uzbek public health institutes, the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases (RSSPMCEMIPD) and the Research Institute of Virology (RIV), are trained in mobile diagnostics of infectious diseases and the handling of the Mobile Lab. The trainings are supplemented by two Field Exercises during which the trainees demonstrate their capability to perform in the Mobile Lab under simulated disease outbreak conditions.

Sustainable laboratory capacities complying with international quality and safety standards are integral for strong health systems and services. The Mobile Labs will allow Uzbekistan to provide complete skilled care in remote regions as well as strengthen the national response capacities in disease outbreak conditions by reducing the analysis time of diagnostic samples. Furthermore, they will provide a great opportunity to increase Uzbekistan"s base of qualified specialists and improve national curricula and teaching methods.

NP25

Development of PCR for differentiation of *F. tularensis subsp. tularensis* and *F. tularensis subsp. holarctica*

Oksana Zlenko[1], Claudia Popp[2], Heiner von Buttlar[2], Anton Gerilovych[3], and Julia Schwarz[2]
1- National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Genetics of microorganisms, Kharkiv, Ukraine; 2- Bundeswehr Institute of Microbiology, Department of Bacteriology and Toxicology, Munich, Germany; 3- National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Kharkiv, Ukraine

Tularemia is a zoonotic infection caused by gram-negative bacterium *Francisella tularensis*. The *F. tularensis* subsp. *tularensis* (A) and *F. tularensis* subsp. *holarctica* (B) are two epidemiologically important types. Type A is the most virulent and present in North America, while less virulent type B is common in Europe and Asia. However, both types can be found in different continents of Northern Hemisphere.

The genome similarity of these subtypes is about 95% (Clarridge, 1996). The MLVA, ERIC, REP, etc. methods admit typing of different isolates. However, they are more time- and cost-consuming then PCR. The most appropriate difference between Type A and B genomes is a 30-bp sequence upstream of a gene encoding an RNA helicase. In silico analysis of 192 *F. tularensis* isolates has shown a complete subspecies conservation of the locus (Johansson, 2000; 2004). However, this 30-bp region is GC-poor and is not an optimal target sequence for a Taq-man probe based qPCR assay.

Thus, we decided to develop a blocker PCR. A blocker is a modified primer that specifically binds to the target DNA and inhibits the polymerase. There are different modifications that could be used for blocker creation, and are applicable both for detection of SNPs and bigger genetic differences.

The PNA (peptide nucleic acid) blocker backbone is built up by N-(2-aminoethyl)-glycine units. It contains no charged phosphate groups, so the binding between PNA and DNA is stronger than between DNAs. This causes a higher melting temperature and has the advantage that the sequence of the blocker can be shorter than that of classical primer (Navarro, 2015 Hong, 2016; Bender, 2018).

As a second approach, we use a T-blocker. It is complementary to both the target DNA sequence and the 3' end of the forward primer. The T-blocker is formed when the 3' end of the forward primer and the 5' end of the blocker bind to each other and their remaining nucleotides bind to the complementary strand of the target DNA forming a structure that does not allow base-pair extension so that no amplification occurs. Additionally, the T-blockers are synthesized with modifications such as lack of a free hydroxyl group at the 3' end to stop polymerase (Orou, 1995; Kim, 2018). We will present first results of the inhibition effects of different blocker concentrations on the detection of *F. tularensis* subsp. *holarctica* via titration curves. Then, these findings are going to be implemented in a probe-based detection PCR.

PP1

Development of fixed phage cocktails in the fight against multidrug-resistant organisms

Andrey A. Filippov, Mikeljon P. Nikolich, Damon W. Ellison, Derese Getnet, and Brett E. Swierczewski
Walter Reed Army Institute of Research, Bacterial Diseases Branch, Silver Spring, MD, USA

Wound infections caused by multidrug resistant (MDR) Gram-negative bacterial pathogens present a persistent challenge for the United States Department of Defense Military Health System (MHS). Antibiotic treatment options are becoming more limited with the proliferation of multidrug resistance and the lack of new antibiotic development. *Pseudomonas aeruginosa* is an important Gram-negative pathogen that causes millions of serious infections globally every year (El Zowalaty et al., 2015) and is a major cause of wound infections, including those seen in complicated combat-related wounds, burns and fractures (Calhoun et al., 2008; Mulcahy et al., 2014). The rapid increase in infections with MDR *P. aeruginosa* is of particular concern as variants have emerged with resistance to carbapenems often used as last line therapeutics and are prevalent in military wounds and burns (El Zowalaty et al., 2015; Calhoun et al., 2008; Keen et al., 2010; Hospenthal et al., 2011). *P. aeruginosa* also forms robust biofilms during infection that are associated with increased antimicrobial tolerance and wound infection persistence (Mulcahy et al., 2014; Akers et al., 2014). The overarching goal of the US Army Bacteriophage Therapeutics program (BT) in the Walter Reed Army Institute of Research Wound Infections Department is to develop durable, off-the-shelf bacteriophage products for the treatment and potential prophylaxis of Gram-negative MDR infections. BT has developed and characterized a panel of therapeutic candidate lytic *P. aeruginosa* phages and employed a rational design approach to create a durable fixed cocktail formulation with lytic activity against 78% of strains in a panel of 100 genomically diverse MDR clinical isolates collected throughout the MHS. The recent success of WRAIR therapeutic phages as treatments in expanded access cases of MDR *P. aeruginosa* infections indicates their potential for combatting MDR organisms.

PP2

Practical assessment of an interdisciplinary bacteriophage delivery pipeline

Silvia Wuerstle[1], Jana Stender[2], Kathrin Rothe[3], Christian Willy[4], Kilian Vogele[5], Patrick Grossmann[5], Jens A. Hammerl[6], and Joachim Bugert[2]
1- School of Medicine, University Hospital Rechts der Isar, Technical University of Munich, Department of Internal Medicine II, Munich, Germany; 2- Institute of Microbiology of Bundeswehr, Munich, Germany; 3- Institute for Medical Microbiology, Immunology and Hygiene, Technical University of Munich, School of Medicine, Munich, Germany; 4- Clinic for Trauma Surgery and Orthopedics, Septic Reconstructive Surgery, Bundeswehrkrankenhaus Berlin, Berlin, Germany; 5- Physics of Synthetic Biological Systems-E14, Physics-Department and ZNN, Technical University of Munich, Munich, Germany; 6- Unit Epidemiology, Zoonoses and Antimicrobial Resistances, Department Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany

Introduction: In the wake of rising antibiotic resistance, bacteriophages (phages) attracted more attention in the treatment of bacterial pneumonia and, more recently, COVID-19 superinfections. Phage manufacturing and delivery not only faces regulatory hurdles, but can be challenging due to its complex and time-sensitive organization.

Aims: (1) To establish a well-coordinated interdisciplinary organizational pipeline for phage delivery prior to quality and safety steps and (2) to assess and increase the pipeline"s efficiency.

Methods: Bacterial isolates as well as clinical data relevant for phage treatment were transferred from a tertiary care hospital to a phage laboratory. Patient data retrieval, effectivity of phage isolation, amplification, and genetic characterization, cumulative time of the single steps of the pipeline, final phage titer and final phage volume at the end of the pipeline were taken into account for the efficiency calculation. Due to varying or non-existent

guidelines for further steps for quality, safety and pharmaceutical preparation in different countries, we focused the pipeline on all steps up to a defined phage volume and titer from *in vitro* phage production.

Results: Different competences required for embedding phage isolation for individual patients were intertwined from an organizational perspective in the presented pipeline. Following pipeline and efficiency equation set-up, the first collection phase of bacterial isolates from tracheal secretions or bronchial lavages from four Intensive Care Units of a tertiary care hospital (25 patients, including 17 Covid-19 patients), revealed a cumulative time up to 538 hours for a suitable phage volume and titer. Following collection phases targeting and adjusting specifically the set points of the most vulnerable component of the pipeline, phage isolation, ultimately increased the pipeline's efficiency by more than twofold. The developed efficiency equation can be accessed via a web application.

Conclusion: We present here a practical approach of a holistic pipeline for phage delivery addressing the individual steps of bacterial characterization, phage isolation, phage characterization, and phage *in vitro* production, as well as the stable interlocking of different institutions and an efficiency assessment for phage laboratories.

PP3

Bacteriophages - Old antiinfectives with new perspectives

Holger Ziehr[1], M. Brack[2], C. Dasenbrock[3], D. Garbe[4], M. Häfner[5], S. Hebecker[1], R. Hertrampf[6], A. Hüser[6], I. Korf[1], B. Lang[7], A. Lakowitz[1], M. Müller[7], C. Rohde[8], A. Ross[1], Q. Schefer[6], C. Seitz[1], M. Stichling[5], W. Wenzel[7], S. Wienecke[1], S. Wienhold[2], C. Willy[5], J. Wittmann[8], and M. Witzenrath[2]

1- Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Braunschweig, Germany; 2- Charité-Universitätsmedizin Berlin (Charité), Department of Infectious Diseases and Pulmonary Medicine, and Division of Pulmonary Inflammation, Berlin, Germany; 3- Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany; 4- Department XXIV Hospital Pharmacy, Bundeswehr Hospital, Berlin, Germany; 5- Department Trauma & Orthopedic Surgery, Septic & Reconstructive Surgery, Research and Treatment Center for Complex Combat Injuries, Bundeswehr Hospital , Berlin, Germany; 6- Charité Research Organisation (CRO), Berlin, Germany; 7- Department XXI Clinical Microbiology & Hospital Hygiene, Bundeswehr Hospital, Berlin, Germany; 8- Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Bacterial pathogens are increasingly developing resistances to all groups of antibiotics. As consequence there are multiple cases of bacterial infection in which available antibiotics are no longer effective. This is the motivation to investigate supplements to classical antibiotic therapy. One promising method is the therapeutic use of bacteriophages.

Two therapeutic phage projects, Phage4Cure and PhagoFlow are actually performed by Fraunhofer ITEM in Braunschweig and its project partners.

Pseudomonas aeruginosa is one of the most frequently bacteria found in the lung of cystic fibrosis patients and leads to more progressive and severe course of disease. Since 2017 the project Phage4Cure is funded by Federal Ministry of Education and Research (BMBF). In this project three *P. aeruginosa* phages were isolated by German Collection of Microorganisms and Cell Cultures (DSMZ), propagated in host cells and purified for application in an inhalable cocktail. The phage API manufacturing is performed in compliance to pharmaceutical quality requirements i.e. Good Manufacturing Practice (GMP). Each phage drug product component is aseptically manufactured at Fraunhofer ITEM. The Phage4Cure phage cocktail will be used in a clinical trials (Phase I, IIa) in 2022. Currently, the manufacturing license extension is filed and waiting for approval.

The DSMZ, Fraunhofer ITEM Braunschweig and the Bundeswehr Hospital Berlin have started the joint project PhagoFlow in 2019, which is publicly funded by the Innovationsfond des Deutschen Bundestages. The aim of this project is to investigate manufacturability and quality requirements for magistral phage preparation in Germany for the treatment of patients with wound infections of the extremities. Magistral phage preparation represent customized formulation of phage cocktails individually tailored for patients infection. Target bacteria are pathogens of priority class 1 and 2 of the WHO list, for which new antibiotics are urgently needed. This project is performed in close alignment with the Federal Institute for Drugs and Medical Devices (BfArM). Currently, manufacturing license is filed and the whole platform-like process sequence for PhagoFlow will be operational from quarter 3 2021.

PP4

Modified Appelmans protocol for in vitro Kp phage host range expansion

Nadine A. Jakob[1], Jana Stender[1], Daniela Friese[1], Jens A. Hammerl[2], Andrey A. Filippov[3], Brett

E. Swierczewski[4], Roman Wölfel[5], and Joachim J. Bugert[1]
1- Bundeswehr Institute of Microbiology, Virology, Munich, Germany; 2- Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany; 3- Walter Reed Army Institute of Research, Emerging Bacterial Infections, Silver Spring, USA; 4- Walter Reed Army Institute of Research, Bacterial Diseases, Silver Spring, USA; 5- Bundeswehr Institute of Microbiology, Institute Director, Munich, Germany

Multi-antibiotic resistant *Klebsiella pneumonia* (Kp), also known as MRGN (multi-resistant gram negatives), one of the consequences of inappropriate use of antibiotics in patients and livestock (One-Health), have led to a high rate of MRGN Kp in patients and the environment. In view of high case fatality rates in patients, alternative therapeutic methods must be considered

Bacteriophages as adjuvant therapeutics might be a suitable approach.

In the phage working group of the Bundeswehr Institute of Microbiology (IMB), we isolate and characterize bacteriophages from wastewater on highly resistant clinical isolates of 3 to 4 MRGN Kp (carbapenem resistance). These Kp isolates show a large variety of capsule types (80+) and are difficult to treat due to the formation of biofilms.

The future use of bacteriophages against MRGN Kp for therapeutic purposes would be more effective using Kp phages with the ability to lyse different capsular types (broad host range).

We currently use a modified "Appelmans protocol" (Ben H. Burrowes et al., 2019), to create phages with a broad host range via in vitro genetic gene transfer between the different phages in targeted phage host combinations. Three phages were selected, combined with eight selected bacterial strains, and grown over multiple cycles in the Varioskan (LUX Multimode microplate reader; Thermo Fisher, Waltham, Massachusetts) at 37°C in 96-well plates overnight. After each cycle, certain wells were pooled together, centrifugated and filtrated (0,2"m). The resulting phage cocktail was then used again to start the next cycle. This experiment is currently in progress.

If phages with extended host range can be identified by whole genome sequence analysis of the products of the modified Applemans protocol, the underlying mechanisms will be used for the deliberate design of GT_ phage with extended host range for therapeutic purposes in our *Phage4-1Health* program.

PP5

Lytic phages from German waste water treatment plants of livestock slaughterhouses and their potential to combat multidrug-resistant P. aeruginosa isolates

Simone Schnehle[1], Claudia Jäckel[1], Mykhailo Savin[2], Silvia Schmoger[1], Annemarie Käsbohrer[1], Ashisch Gadicherla[1], Judith Kreyenschmidt[2], and Jens A. Hammerl[1]
1- German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany; 2- University of Bonn, Institute of Animal Sciences, Bonn, Germany

Questions: Pseudomonads are Gram-negative bacteria of the Pseudomonadaceae for which currently 191 valid species have been reported. The species *P. aeruginosa* has a high impact in clinical settings, as they efficiently exchange DNA (i.e. resistance plasmids) with other bacteria. Antimicrobial resistance of multidrug-resistant isolates is multifactorial and based on chromosomal and extrachromosomal factors. Due to the steadily increasing number of multidrug-resistant *Pseudomonas* isolates in clinical settings, efficient strategies for control of these bacteria are needed. As bacteriophages have been reported as suitable alternatives for the treatment of multidrug-resistant isolates, this study aimed to determine the occurrence of lytic phages from waste water treatment plants of poultry and pig slaughterhouses.

Methods: The recovered phages were characterized in their phenotypic (host rang, plaque formation, TEM) and genotypic properties (WGS) to determine their potential for the treatment of *Pseudomonas* spp. bacteria.

Results: In general, all recovered phages exhibited a highly restricted host range, which is limited to the species *P. aeruginosa*. However, the spectrum and the number of lysed isolates vary substantially, and provide thus a good basis for the development of a phage cocktail with broad activity. Overall, the phages were shown to be stable under a wide range of pH and temperature conditions. On the basis of their virion morphology, the phages were allocated to different groups of *Pseudomonas* phages. WGS and bioinformatics analyses revealed detailed information on their phylogenetic relationship to different virus-genera. Furthermore, sequence analyses indicated that the recovered phages are suitable for therapeutic issues as they did not carry antimicrobial resistance and/or virulence determinants.

Conclusions: On the basis of the prevailing results, lytic phages can be used to combat *P. aeruginosa* with high efficacy. However, in order to develop a broad active and highly efficient phage compound that prevents development of phage resistant bacterial isolates, the cocktails need to be carefully composed.

PP6

Restoration of antibiotic susceptibility in *Klebsiella pneumoniae* using capsule type specific non-lytic bacteriophages

Jana Stender[1], Jens A. Hammerl[2], Daniela Friese[1], Roman Wölfel[1], and Joachim J. Bugert[1]
1- Bundeswehr Institute of Microbiology, Virology, Munich, Germany; 2- Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

The alarming rise of multi-antibiotics resistant bacteria is probably the greatest health challenge of our time from a one-health perspective, with multiresistant organisms detectable in hospitals, livestock and in the environment. In terms of medical treatment challenges, the focus is on ESKAPEE organisms, which cause the highest case fatality.

The use of bacteriophages (phages) is now considered again as an alternative treatment option in Western Europe and America. In comparison to Eastern European countries with well-established phage therapy guidelines, there are still concerns in the West regarding the use of phages as a standardized treatment.

Combination treatment of phages followed by antibiotics is seen more favourably and is based on the hypothesis that some phage can restore antibiotic susceptibility in MRGN bacteria.

This project aims to test this hypothesis using wzi capsule typed non-lytic phages on 4MRGN *Klebsiella pneumoniae* (Kp) to restore antibiotic susceptibility. The capsule of Kp consists of capsule-polysaccharides (CPS), which represent the first receptor for the phage. The second receptor is located on the cell surface and is strain dependent. Consequently, a phage specific for a certain capsule type (CT) does not necessarily bind to the second receptor to lyse the Kp, but the loss of CPS due to phage depolymerase activity may make MRGN Kp susceptible to antibiotics, that depend on efficient crossing of the CPS barrier.

Two Kp strains, 1762o and 4409, showed CT K55/K34 and were identified as 4MRGN and 3MRGN, respectively. Phage Muc 275 was isolated on 4409, causing plaques with a halo, but does not affect the growth kinetic of 1762o, of the same CT. Muc 275 fulfils the set requirements for this study, as it is specific for the CT in question but does not lyse Kp 1762o.

The growth kinetics under antibiotic treatment were compared under different phage infection settings. The results showed a significantly decreased growth ability for the combination of capsule specific non-lytic phage (Muc 275) and several antibiotics on 1762o, compared to the non-phage treated control.

Future studies will be conducted to show if the same results can be achieved using isolated depolymerases from Muc 275.

PP7

Study of the CRISPR/CAS system of the Pseudomonas aeruginosa DSM 50071 strain for screening of phages and plasmids

Lilia A. Stepanenko, Yuri Pavlovich Dzhioev, Andrey Y. Borisenko, Nadezhda A. Arefieva, Vladimir I. Zlobin, and Igor V. Malov
Irkutsk State Medical University, Irkutsk, Russian Federation

Pseudomonas aeruginosa in the modern world is one of the main causes of opportunistic and nosocomial infections. The emergence of strains with multiple antibiotic resistance is a global problem in clinical medicine. The study of the CRISPR/Cas system in bacteria capable of cutting out and incorporating nucleotide sequences from recently discovered foreign DNA (phages and plasmids) into their structure is the basis for the development of new antibacterial treatments.

Materials and methods: The sequence of *Pseudomonas aeruginosa* strain DSM 50071 (NZ_CP012001.1) was downloaded from the NCBI databases and analyzed using bioinformatics software.

Results and discussion: This strain was first described in 1872 by Schreter. It has a genome size of about 6.3 MB, circular DNA consists of 6317050 bp. and contains 5857 genes. This is a type strain, i.e. it has all the genotypic and phenotypic properties characteristic of this species. In the structure of the CRISPR/Cas system of the strain, three CRISPR loci were found, one group of pas genes, characteristic of type I CRISPR / Cas systems, subtype I-F. CRISPR cassettes ranged in size from 807 to 1047 bp. The CRISPR cassettes contained 28 bp repeats separated by spacers (31 to 33 bp). In CRISPR cassettes 2 and 3, complete identification of spacers to protospacers of phages specific for bacteria of the Pseudomonadaceae family, isolated, most often, from the lungs of patients with bronchiectasis, as well as from hospitals and water bodies, was established. Spacer 2 CRISPR-1 fully corresponds to the plasmid of the protospacer pJHX613 Pseudomonas aeruginosa E6130952, which is a pan-resistant strain isolated from the sputum of a patient with respiratory failure, as well as the plasmid pKPN-704 Klebsiella pneumoniae KPNIH39, isolated from a patient with pneumonia. In CRISPR-3, Sp.9 is sim-

ilar to the protospacer of the plasmid pEC743_4 of E. coli Ecol_743 isolated from a patient with intestinal infection, Sp.10, to the plasmid BH9 of the Pseudomonas aeruginosa pBH6 strain, which is resistant to carbapenem. These studies provide information on the adaptive capabilities of this strain and, on the basis of this, implement approaches to the creation of targeted phage therapy.

Index of authors

Click on the abstract number to jump to a particular abstract!

Abstract numbers are composed of a first letter (A-P), indicating the session to which the presentation has been assigned, and a serial number. Poster presentations are indicated by a second letter (P). Abstract numbers of the presenting author are printed in boldface type.