# Program at a glance (Part I)

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## Sunday, October 28

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<tr>
<td>16:00</td>
<td>The Antimicrobial Drug Development Pipeline</td>
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<td>18:00</td>
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<td>18:00</td>
<td>Welcome Reception at the Reception Hall</td>
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## Monday, October 29

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<tr>
<td>08:30</td>
<td>CRISPR/Cas Genome Editing — Opportunities and Concerns</td>
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<td>Vaccine Progress and New Options</td>
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<td>15:30</td>
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<tr>
<td>16:00</td>
<td>New Approaches to Antiviral and Antibiotic Drug Discovery</td>
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<td>18:00</td>
<td>Smallpox in the Post-Eradication Era</td>
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<td>19:00</td>
<td>Conference Dinner at Eventstadel, Moosach (Wasserburg)</td>
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Program at a glance (Part II)

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### Tuesday, October 30

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<tr>
<td>08:30-10:30</td>
<td>Recent Advances in Targeted and Open View Diagnostic Procedures</td>
<td>Secrets Hidden in the Depth of Genomes</td>
<td>Poster Exhibition</td>
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<td>11:00-12:30</td>
<td>Strategies to Counter Biological Risks</td>
<td>Innovative Tools for Medical Bio-Reconnaissance</td>
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<tr>
<td>14:00-15:30</td>
<td>Educational session #1: Ticks and Tick-Borne Encephalitis</td>
<td>Poster Exhibition</td>
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</tr>
<tr>
<td>16:00-16:15</td>
<td>Poster Award Ceremony</td>
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<tr>
<td>16:15-18:15</td>
<td>Global Partners in Biosecurity</td>
<td>Educational session #2: Case Reports / Interactive Voting Session</td>
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### Wednesday, October 31

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<tr>
<td>08:30-10:30</td>
<td>Zoonotic Diseases: A Global Health Problem</td>
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<tr>
<td>11:00-13:00</td>
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The development of new antimicrobials is one of the big issues in medical countermeasures against biological threat agents. In this presentation a general overview of the drug development process, different intervention strategies, and specific drug development case studies will be given. An example of translating an academic idea into a drug candidate from the field of innate immunity will be used for illustration. An additional example will highlight the incubation of an academic idea for a novel antibiotic therapy, which is not yet partnered to industry. The development pipeline will be shown as a process transforming basic research into novel drug candidates.

In order to manage the process and to bring together experts from academia and pharmaceutical industry, the Lead Discovery Centre (LDC) has been established in 2008 as a company under the umbrella of the Max Planck Society. LDC’s academic network represents thousands of scientists and a large pipeline of novel ideas for treating diseases with unmet medical need. LDC has established a flexible, industry-type drug discovery infrastructure, where innovative early stage projects are transformed into attractive licensing opportunities. Its innovative bottom-up drug discovery approach is to start from basic research and to finally find a therapeutic application for a target- or mechanism of interest. This approach seems to be more efficient than the traditional pharma best practice of searching for the “ideal” target within a pre-defined therapeutic area.
to connect and mine the data, open entirely new possibilities to design effective biodefense strategies. Also the possibilities provided through targeted manipulations of the genome contribute new avenues of thought. However, in each case we need to distinguish between possibilities and potential risks which requires the evaluation of the ethical values that might be affected.

Through the CRISPR/Cas technology it might become possible to design effective biodefense strategies through e.g. gene drive but what is the risk we are willing to accept? Furthermore, editing of the human germline might become possible and could be used for self-enhancement approaches generating in the future super soldiers. Is that an avenue we ever want to consider? In light of big data, we hope that it will be possible to optimize treatment for an individual person, but what are the limits that we feel should be considered?

In each of these developments, we are confronted with advantages but also major challenges and it is important to consider ethical values such as human dignity, self-determination, responsibility and solidarity to decide on future directions.

Focus Session

Management of Unusual Infectious Disease Outbreaks

Chairs: G. Grass (DEU) and L. Baillie (GBR)

CO 01
Unprecedented pneumonic plague outbreak, Madagascar 2017

M Rajerison¹, V Andrianaivoarimanana¹, S Rahelimirina¹, B Ramasindrazana¹, JL Rakotomanahary¹, S Rahajandraibe¹, R Randremanana², V Rasolofo³, V Cauchemez⁴, L Baril², M Ratsitorahina⁵, and A Spiegel³

¹- Institut Pasteur de Madagascar, Plague Unit, Antananarivo, Madagascar; 2- Institut Pasteur de Madagascar, Epidemiology and Clinical Research Unit, Antananarivo, Madagascar; 3- Institut Pasteur de Madagascar, Direction, Antananarivo, Madagascar; 4- Institut Pasteur, Mathematical Modelling of Infectious Diseases Unit, Paris, France; 5- Ministry of Public Health, Directorate of Health and Epidemiological Surveillance, Antananarivo, Madagascar

Plague continues to threaten the Madagascan population. Its pneumonic form poses a severe public health risk given the easily person to person transmission and the high mortality rate in case of delayed treatment. This paper described the onset of an unprecedented pneumonic plague (PP) outbreak, its epidemiological characteristics and transmission dynamics.

Besides the century of studies undertaken on Madagascan plague allowing to identify its period of occurrence, endemic area and clinical characteristic, the beginning of plague season is always brutal and plague infection was only identified after successive death in the family or village. For the PP outbreak, an alert was done on September 11, 2017 through the IPM and MoH fever sentinel surveillance. It indicated a health agent presented respiratory distress, fever and admitted at the regional hospital the day before. An investigation by phone allowed to know his previous contact with patient presented unknown disease which evolved rapidly to his death. This information constitutes an alert for the initiation of the investigation, allowing us to identify the case 0, the secondary cases and contact identification. During the 15 days elapsed time between the 1st identified case and the case 0, 5 deaths were identified and were considered as source of this large PP outbreak. In total, 2,414 clinically-suspected plague cases were reported (01/08-26/11/2017), of which 1,878 (78%) were PP cases, 395 (16%) BP cases, 1 septicemic case, and 140 (6%) had unspecified clinical form, with laboratory results available for 99.6% of them. Among notified PP cases, 386 (21%) cases were probable (P) and 32 (2%) were confirmed (P); among notified BP cases, 73 (18%) were P and 66 (17%) were C. The CFR was 25%, 8%, and 5% among C, P and suspected PP cases, respectively. It followed a similar trend in BP cases (C: 24%, P: 6%, suspected: 2%). PP hotspots were mainly identified in Antananarivo, the capital city and Toamasina, the main seaport. All 50 isolated Yersinia pestis strains were susceptible to the tested antibiotics.

This predominantly urban plague epidemic was characterized by a large volume of notifications in two major urban areas and an unusually high proportion of pneumonic forms, with only 23% of them having ≥1 positive laboratory test. Lessons identified in this epidemic are critical to improve response to future plague outbreaks.
CO 02
Epidemiological review of *Francisella tularensis*: A case study in the complications of dual diagnoses

R Stidham, RL von Tersch, DB Freeman, PJ Sullivan, and S Tostenson
US Department of Defense, US Army, Public Health Command Central, Joint Base San Antonio (JBSA)-Fort Sam Houston, TX, USA

Tularemia is a rare but potentially fatal disease that develops in numerous wild and domestic animals, including lagomorphs, rodents, cats, and humans. *Francisella tularensis* bacterium was identified while a rabies specimen was in the process of being submitted to the US Department of Defense (DoD) U.S. Army Public Health Command Central (PHC-C), Food Analysis and Diagnostic Laboratory (FADL) by veterinary personnel at Fort Riley, Kansas. Epidemiologic characteristics of tularemia, *F. tularensis* as an organism of military interest, potential laboratory management to *F. tularensis*, and clinical findings on a case of feline tularemia are discussed. This presentation provides insight on how veterinarian staff and laboratory personnel can clinically manage esoteric, unexplained, or questionable pet death examinations. It further raises questions as to whether or not dead animals should be treated as sentinels and be pre-screened for select agents, especially in instances of dual diagnoses. This case epitomized the essence of the One Health attitude in the high level of patient care that can be achieved when veterinary personnel actively foster working relationships with their local public health counterparts.

CO 03
Utility and readiness of mobile bio-reconnaissance laboratories for biological threat and outbreak investigations

P Matero¹, A Parsons², M Adams³, and KB Yeh⁴
1- Aatos Consulting & Training, Vantaa, Finland; 2- BIA Diagnostics, Colchester, US; 3- GSS Health, Baltimore, US; 4- MRI Global, Gaithersburg, US

Mobile bio-reconnaissance laboratories have long been used by military and defense, law enforcement, environmental monitoring, and health related agencies. Among the purposes of deploying such laboratories are early warning, forward reconnaissance, and on-site investigation. With the advent of technology that has enabled the use of diagnostic instruments in the field, in addition to transportation capabilities to rapidly deliver these assets and services, mobile and field units have served to further extend networks of existing, fixed laboratories. Our presentation discusses case histories of example mobile bio-reconnaissance laboratories that have been recently deployed for biological threat and outbreak investigations. Examples include mobile bio-reconnaissance laboratories used by the US military, the Finnish defense for countering biological threats (i.e. WMD) and several used in response to the 2014-2016 Ebola virus outbreak in West Africa such as the Bundeswehr fielded laboratory. Our discussion includes a comparison of missions, operations, and capabilities among these examples mobile and field laboratories. These laboratories often possess detection and diagnostic capabilities, dedicated working areas and specific equipment are required for sample handling, performing analyses, packing and shipment, waste disposal, decontamination, and associated communication and reporting. Given the often-remote locations to which these laboratories are deployed, they must incorporate a high degree of self-sufficiency, particularly with regard to utilities, and integral biosafety and biosecurity measures. Experienced scientists performing similar technical tasks in fixed laboratories can be employed to support and refine these operations. Mobile bio-reconnaissance laboratories are usually national level assets that require ongoing funding for maintenance, equipping, resupply and training of personnel in order to maintain a readiness posture for their intended missions. The cost and sustainment challenges of operating and maintaining these important assets are addressed in lessons learned to provide recommendations for their practical use and deployment.

CO 04
Performance of Public Health in running a foodborne bioterrorism situation

PT Silva
Escola de Serviço de Saúde Militar, Direção de Saúde Militar, Lisboa, Portugal

Holding the consumer harmless from biological, chemical or physical threats that may be caused by foodstuffs prepared and/or ingested according to intended use is the main goal of Food Safety. In the overall context of food defense, threats that are intentionally introduced to alter foodstuffs compromising public health and potentially causing social and economic disruption should be particularly considered. The disaster events involving biological agents require the establishment of surveillance systems and response mechanisms, constituting a particular concern of government agencies and of society in general. These situations require the cooperation
between several areas of expertise that are usually represented in different state departments and among which it is necessary to establish communication channels and coordination systems. Governments, as well as public and private organizations, should be aware of the need to develop food defense plans in order to prevent and respond to food bioterrorism attacks. These plans shall include prevention, preparation and response measures to be developed in the event of an attack situation:

**Prevention:** relies on the careful application of existing food security programs and the implementation of weighted security measures, based on the recognition of vulnerabilities of food systems.

**Preparation:** depends to a large extent on vigilance plans developed and implemented long before the occurrence of a food bioterrorism situation.

**Response:** it should be fast and effective, so as to allow prompt resolution of the detected problem and mitigation of its impact. A correct articulation between the different public or private players involved in the response and a deep knowledge of the roles played by each of them in the course of the outbreak.

Foodborne bioterrorism must therefore be considered a serious threat potentially causing severe public, economic or social health problems. Changes in the production, marketing and consumption of foodstuffs, as well as the emergence and re-emergence of numerous food hazards present a huge challenge for societies and require the establishment of new and more effective contingency plans for emergencies of this kind.

**CO 05**

Lessons from 2015 MERS attack and changes thereafter in Korea

NT Lee
Korea University, Seoul, Korea

Nowadays the possibility of biocidents is increasing compared to the past, due to the frequent emergence of pandemic diseases along with bioterrorism and accidental release of infectious pathogens from laboratories. Furthermore, it is considered that the vulnerability to biological attack in Korea is relatively higher due to the North Korea’s biological weapons. In this regard, fostering biodefense capability is very important to Korean government. One of the recent cases that had put Korea’s biodefense capability on the test stand was 2015 MERS. It was initially flowed in from Middle East Country by a Korean traveler and caused 36 death toll out of 186 confirmed cases in three months. The outbreak was the largest pandemic which Korean people have ever experienced during the last decade, in terms of economic depression and social panic.

Based on the epicurve, the basic reproduction number of 2015 MERS was estimated at 11.5, which indicates that the infection rate of 2015 MERS was uniquely higher compared to other recent pandemics such as New Flu and SARS.

In the course of responding to the 2015 MERS, Koreans learned that the initial stage of biorisk management was rather unsuccessful due to the following reasons: lack of knowledge and experience of the physicians about the characteristics of MERS-CoV, delayed confirmation of the first index case, poor sharing of medical information between hospitals and general public, poor application of quarantine criteria, and delayed use of in vitro diagnosis kit. However, Korean government after paying a high price, could subdue the spread of the MERS and gain priceless lessons from its attack, and thereafter have been reflecting such lessons in the disease defense strategies not only for civilian but also for soldiers.

Therefore, in this conference, lessons from 2015 MERS attack and changes after that will be introduced, mainly focusing on the following issues: revision of national disease prevention law and systems, criteria change of the facilities of medical institutes, upgrade of medical strategic material stockpiling, creation of bioterrorism drills and exercises, revision of patient visiting culture, etc.

**CO 06**

The Tunisian mobile laboratory: Conceptions and missions

M Ben Moussa1, H Naija1, F Barguellii2, M Diehl2, G Zikeli2, S Handrick2, and R Wölfel2
1- Department of Microbiology, Military Hospital of Tunisia, Tunis, Tunisia; 2- Bundeswehr Institute of Microbiology, Munich, Germany

**Background:** Epidemic-prone infectious diseases still pose a serious public health threat in many countries and the risk of the spread of infectious diseases has increased. Many biological agents, which belong to the high and highest risk groups 3 and 4, can cause a hazardous and serious outbreaks that can pose serious challenges to local health services and international organizations.

In 2017 and in coordination with the Bundeswehr Institute of Microbiology in Munich (IMB) and the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ); a biological mobile laboratory level 3/4 is delivered to the military health to support and increase the military and national capacities in Tunisia to respond to infectious disease outbreaks.
and strengthen the collaboration between scientists.

**Conception and missions:** The Tunisian Mobile Laboratory (TMB) is dedicated to high-quality molecular and serology diagnostics of groups 3/4 pathogens, to be deployable in the sites of outbreaks and to conduct interepidemic research projects on infectious diseases.

To accomplish all these tasks; scientists and technicians experienced in infectious diseases diagnostics, molecular biology and research from the military hospital of Tunis, Pasteur Institute of Tunis and Charles Nicolle Hospital; are trained in workshops by the IMB teams.

After that; extensive range of laboratory equipment was procured.

The principal missions of the TML are the respond to diagnostic field missions in the site of outbreaks of epidemic infectious diseases upon request, strength scientific research on infectious diseases that are endemic in Tunisia and the diagnostic capabilities of the microbiology laboratory in the military hospital of Tunis.

**Conclusion:** The TML can be an integral part of an epidemiological surveillance system because of its ability to bring the diagnosis closer to the infectious site and shorten the time to obtain the result. Its role is complementary to that of the P3 / P4 fixed laboratories, which is absent in Tunisia; thus it can intervene quickly on the site of a presumed hearth, allow a first identification of the pathogen and facilitate the implementation of measures of containment pending a more precise confirmation by a P3 / P4 laboratory in the maximum respect of the regulations of biosecurity.

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**DO 01**

**Evaluation of a standard operating procedure for antimicrobial susceptibility testing of highly pathogenic bacteria – An inter-laboratory study**

A Tscherne¹, E Georgi¹, MC Elschner², A Fasanella³, SL Feruglio⁴, R Grunow⁵, C Hinz¹, D Jacob⁵, TB Johansen⁴, T Boskani⁸, V Manzulli³, S Nuncio⁷, A Pelerito⁷, K Szulowski⁸, N Schürch⁹, S Thomann⁹, H Tomaso², T Wahab⁶, I Wojciech⁸, L Zöller¹⁰, and S Zange¹

¹- Bundeswehr Institute of Microbiology, Central Diagnostic Unit, Munich, Germany; ²- Friedrich-Löffler-Institut, Jena, Germany; ³- Istituto zooprofilattico sperimentale della puglia e della basilicata, Foggia, Italy; ⁴- National Veterinary Institute, Oslo, Norway; ⁵- Robert Koch Institute, Centre for Biological Threats and Special Pathogens (ZBS 2), Berlin, Germany; ⁶- Public Health Agency of Sweden, Stockholm, Sweden; ⁷- National Institute of Health, Lisboa, Portugal; ⁸- National Veterinary Research Institute, Palau, Poland; ⁹- Laboratory of Spiez, Spiez, Switzerland; ¹⁰- Bundeswehr Institute of Microbiology, National Consultant Laboratory for Brucella, Munich, Germany

Introduction: The EU funded joint action EMERGE “Efficient response to highly dangerous and emerging pathogens at EU level” fosters a network of 40 European laboratories with diagnostic capabilities for highly pathogenic bacteria and viruses. Herein, one working group aims to improve standard operation procedures (SOPs) for antimicrobial susceptibility testing (AST) of risk group 3 bacteria, as it is a crucial task in terms of therapy success or failure. No European breakpoints are available for these agents yet; therefore, American guidelines and procedures were adopted. However, different problems and issues within these guidelines make it necessary to adapt the procedures and breakpoints for implementation of European standards.

Material and methods: Broth microdilution method (BMD) was chosen to determine the minimal inhibitory concentration (MIC) towards antibiotics typically used for therapy of these agents. Based on experiences from a former EU project, customized microdilution plates containing 20 antibiotics in log2 dilutions were designed. A comprehensive SOP including a reading guide was developed and project partners were trained on the method. Finally nine laboratories participated in an inter laboratory trial to validate the SOP. Four clinical isolates of *Bacillus anthracis*, *Brucella melitensis*, *Francisella tularensis* ssp. *holarctica* and *Burkholderia pseudomallei*, one of each species, were tested with 10 replicates per site. MIC value ranges were defined based on the mode of each species/substance combination.

Results: 58 datasets (species/medium/substance combinations) consisting of more than 5,000 single data points were analyzed. Inter- and intra-laboratory variation was low resulting in a narrow
distribution of log2 transformed MIC values. Even laboratories less experienced with the BMD method achieved comparable results using the SOPs developed by the network.

Conclusion: Standardized procedures are important to compare diagnostic results between laboratories. Due to the SOP and reading guide, an improvement of observer-dependent determination of MIC could be seen. However, single outliers suggest the necessity of further practical trainings. In a next step, the new SOPs may serve as basis for European guidelines and will be used to test strain collections of each participating institute to generate epidemiological cut-off values (ECOFFs), which are the basis for determination or modification of breakpoints.

**DO 02**

*Burkholderia ubonensis* meropenem resistance: A tale of a near neighbor of *Burkholderia pseudomallei*

N Somprasong¹, CM Hall², DM Wagner², P Keim², and HP Schweizer¹

¹- University of Florida, Emerging Pathogens Institute, Gainesville, USA; ²- Northern Arizona University, The Pathogen and Microbiome Institute, Flagstaff, USA

*B. pseudomallei* (Bp) is a biothreat agent and causes melioidosis. Bp infections are difficult to treat due to the bacterium’s intrinsic or acquired antibiotic resistance. Melioidosis treatment requires an acute phase therapy limited mostly to β-lactam antibiotics such as ceftazidime and carbapenems. Concern is that Bp could enhance its already significant drug resistance repertoire by acquisition of DNA from drug resistant near neighbor species.

A bacterium that is commonly co-isolated from environments where Bp is present is B. *ubonensis* (Bu) whose biology is not yet well understood. Unlike Bp, meropenem resistance (MEMr) is not uncommon in Bu, but the underlying mechanisms are unknown. In an attempt to understand MEMr in Bu, we used molecular tools to analyze BP8955, a MEMr (MIC ≥32 µg/ml) Bu soil isolate from Puerto Rico. Although Bu, like other *Burkholderia* spp., encodes a Class D β-lactamase that constitutes clinically significant OXA carbapenemases in diverse Gram-negative pathogens, Bu did not express carbapenemase activity as assessed by the Carba NP test. Unlike Bp, Bu encodes two Class A β-lactamas enes, PenA and PenB. Bu and Bp penA exhibit the same chromosomal organization, but a closer analysis of the Bu PenA amino acid sequence revealed that the protein is missing its active site serine and lacks key residues in other β-lactamase Ambler consensus motifs. PenA therefore likely lacks β-lactamase activity. PenB contains the active site serine and all Ambler motifs, and its genetic association with a transcriptional regulator suggests that it is an inducible β-lactamase. Bu also encodes an AmpC homolog, which in Enterobacteriaceae and a few other bacteria is an important chromosomally encoded and inducible cephalosporinase. A random transposon mutagenesis approach was employed to identify MEMr determinants. Transformants with reduced MEMr (growth on MEM ≤8 µg/ml) were obtained at a frequency of 0.28% (10 out of 3,515 analyzed). Transposon insertion sites were determined by sequencing chromosome-transposon junction sequences. Mutants exemplifying opposite sides of the MEM susceptibility (MEMs) spectrum - MICs of 8 and 1.5 µg/ml – were further analyzed. Two of the lesser MEMs mutants contained insertions in BamC, an outer membrane (OM) assembly apparatus accessory protein. Its knockout likely affects OM permeability. Two highly MEMs mutants contained transposon insertions in Slt, a soluble transglycosylase, and NagZ, a β-N-acetylglucosaminidase. In other bacteria, Slt and NagZ homologs are required for β-lactamase induction. Our data show that Bu β-lactam resistance is complex; similar, but not the same as in Bp, where only PenA is clinically significant. The repertoire of β-lactamases in Bu is more similar to *B. cepacia* complex than Bp complex organisms. Studies with near neighbor species are informative about the diversity of antimicrobial resistance in *Burkholderia* species.

**DO 03**

Evaluation of in vitro antimicrobial susceptibility of *Bacillus anthracis* strains isolated during anthrax outbreaks in Italy from 1984 to 2017

V Manzulli¹, M Caruso¹, L Serrecchia¹, D Galante¹, A Donatiello¹, V Rondinone³, S Zange², A Tscherner², A Parisi¹, and A Fasanella¹

¹- Instituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Anthrax Reference Institute of Italy, Foggia, Italy; ²- Bundeswehr Institute of Microbiology, Munich, Germany

Anthrax, caused by *Bacillus anthracis*, is a non-contagious infectious disease that affects a wide range of animal species (primarily ruminant herbivores), as well as humans. Due to the often fatal outcome of human cases, the quick administration of definitely effective antimicrobials is crucial either as prophylaxis or for the therapy of clinical cases. In this study, a total of 110 *B. anthracis* strains, temporally, geographically and genetically diverse, isolated during anthrax outbreaks in Italy from 1984 to 2017, were screened for their susceptibility...
towards sixteen clinically relevant antimicrobial agents using the broth microdilution method. The strains were isolated from various matrices (human, animal and environmental) and were representative of thirty distinct genotypes identified by MLVA 15 loci. The following antimicrobials were tested: gentamicin, ceftriaxone, streptomycin, penicillin G, clindamycin, chloramphenicol, vancomycin, linezolid, cefotaxime, tetracycline, erythromycin, rifampicin, amoxicillin, ciprofloxacin, doxycycline and trimethoprim. The lowest concentration of each antibiotic that prevented bacterial growth represented the minimal inhibitory concentration (MIC). All isolates were susceptible towards most of the tested antimicrobials with the exception of trimethoprim for which all of them showed high MIC-values. For ceftriaxone and cefotaxime an intermediate level of susceptibility was recorded. Although the Centers for Disease Control and Prevention recommend the use of doxycycline, ciprofloxacin, penicillin G and amoxicillin for the treatment of human cases and for post exposure prophylaxis to anthrax spores, this study shows a high degree of in vitro susceptibility of \textit{B. anthracis} to many other antimicrobials, thus suggesting the possibility of alternative choice for prophylaxis and therapy of infections. Monitoring the antimicrobial susceptibility of \textit{B. anthracis} is very important for the management of anthrax infections in order to choose the best therapeutic strategy and to identify possible resistant clones.

**Results:** By sequence data, the majority of isolates belonged to the East Mediterranean clade, while the remaining belonged to the African clade. All isolates were susceptible for tested antibiotics, except for rifampicin, where phenotypical resistance was detected in all isolates based on broth microdilution method, and in four isolates based on gradient strip testing. In contrast, screening of the gene involved in rifampicin resistance (\textit{rpoB}) did not reveal any resistance driving mutations, indicating overestimation of resistance based on phenotypical results. **Conclusion:** Our data show that human brucellosis in Norway is linked to travelling and migration from the Middle East, Asia or Africa, and that travel history and genetic epidemiological sequence data harmonize well. Based on our results, occurrence of antibiotic resistance in \textit{B. melitensis} is low, and current Norwegian empirical guidelines for treatment are valid. Although the number of reported cases in Norway is low, there is a reason to keep awareness about this disease due to migration patterns and cross border movements.

**DO 04**
Whole-genome sequencing and antimicrobial resistance of \textit{Brucella melitensis} from a Norwegian perspective

SL Feruglio, TB Johansen, L Scheffer, VK Jensen, and J Bohlin
1- Norwegian Institute of Public Health, Department of Zoonotic, Food- and Waterborne Infections, Oslo, Norway; 2- Hanze University of Applied Sciences, Groningen, The Netherlands; 3- Norwegian Institute of Public Health, Department of Bacteriology, Oslo, Norway; 4- Norwegian Institute of Public Health, Department of Bioinformatics, Oslo, Norway

**Background:** Brucellosis is a zoonotic infection, transmitted to humans by direct contact with infected animals or consumption of infected animal products, especially unpasteurized milk products. The aim of this study was to explore all \textit{Brucella melitensis} isolates collected in Norway from 1999 to 2016 in relation to origin of infection and antimicrobial resistance.

**Methods:** A total of 23 isolates were analysed by whole-genome sequencing and compared with selected \textit{B. melitensis} genomes available from NCBI. Additionally, SNP analysis in antibiotic resistance determining genes was performed, and compared with phenotypical antibiotic resistance patterns determined by broth microdilution and/or gradient strip test. Travel history was obtained from registered data in the Norwegian Surveillance System for Communicable Diseases.

**Conclusion:** When compared to world-wide data, the majority of the isolates were susceptible to most antibiotics, especially amoxicillin, ciprofloxacin, doxycycline, penicillin G, clindamycin, chloramphenicol, vancomycin, linezolid, cefotaxime, tetracycline, erythromycin, rifampicin, amoxicillin, ciprofloxacin, doxycycline and trimethoprim. The lowest concentration of each antibiotic that prevented bacterial growth represented the minimal inhibitory concentration (MIC). All isolates were susceptible towards most of the tested antimicrobials with the exception of trimethoprim for which all of them showed high MIC-values. For ceftriaxone and cefotaxime an intermediate level of susceptibility was recorded. Although the Centers for Disease Control and Prevention recommend the use of doxycycline, ciprofloxacin, penicillin G and amoxicillin for the treatment of human cases and for post exposure prophylaxis to anthrax spores, this study shows a high degree of in vitro susceptibility of \textit{B. anthracis} to many other antimicrobials, thus suggesting the possibility of alternative choice for prophylaxis and therapy of infections. Monitoring the antimicrobial susceptibility of \textit{B. anthracis} is very important for the management of anthrax infections in order to choose the best therapeutic strategy and to identify possible resistant clones.

**DO 05**
DNA microarrays for the culture-independent detection of extended spectrum beta-lactamase genes and bacterial pathogens

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Antibiotic resistance has become an important issue over the last years and especially antibiotic resistant gram-negative bacteria are in the focus. Multidrug resistant bacteria are of great interest due to the range of negative health effects caused by them in human and animals alike. Extended spectrum beta-lactamase (ESBL) producing bacteria are one example thereof, which convey resistance against several \beta-lactam antibiotics. An important gene class coding for ESBLs are CTX-M genes, which are currently among the most frequently detected resistance genes worldwide. Therefore, they are an appropriate target for detection of antibiotic resistant bacteria via DNA-based methods. Currently, culture-dependent techniques are
a bottleneck for prompt risk assessment due to long incubation times. Here, a DNA microarray based on heterogeneous asymmetric recombinase polymerase amplification (haRPA, Kunze et al.) was adapted to detect the ESBL gene CTX-M clusters 1 and 9 in different bacterial species and the bacteria Klebsiella pneumoniae and Pseudomonas aeruginosa. The haRPA allows sensitive and rapid quantification and qualification of specific DNA sequences in less than 1 h after DNA extraction. In individual measurements, the different gene clusters for the respective antibiotic resistance gene were detected in K. pneumoniae and Escherichia coli. Additionally, K. pneumoniae and P. aeruginosa as bacterial species were also identified in individual measurements. One big advantage of the haRPA is the detection of several different DNA sequences simultaneously by multiplexing. With an additional viability haRPA, using propidium monoazide treatment prior to haRPA (Kober et al.), screening for risk factors and their potential for infection in environmental samples is possible on one single microarray chip. The combination of multiplex and viability haRPA is a promising tool that will allow for faster screening of environmental samples for the presence of resistance genes and pathogenic bacteria.

References:

**EO 01**
**Biodefence vaccines, future prospects and current challenges**

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Vaccination is the most cost effective form of mass protection. The Smallpox vaccine campaign demonstrated the power of this approach in that eliminated the disease from the human race. While there is no doubting their efficacy, developing vaccines to meet current known infectious disease threats is not trivial with costs of between US$800-1.8 billion and timelines in the order of 10-15 years. Development of biodefence vaccines is even more challenging given the circumstances under which an individual is likely to encounter the pathogen. While vaccines to counter bio-threat agents such as Smallpox (Dryvax®) and Anthrax (BioThrax®) exist, concerns over their reactogenicity and immunogenicity has spurred the development of more defined (In-vaccine: modified Vaccinia Ankara vaccine) and immunogenic (NuThrax™: Anthrax Vaccine with CPG 7909 Adjuvant) next generation variants. The development of these modified products has taken many years due in part to an inability to perform human efficacy trials due to a lack of suitable study populations (Smallpox) or for ethical reasons. To address this issue the US FDA introduced the Animal rule under which products can be licensed for human use if they demonstrate efficacy in suitable animal models.

While technologically advanced countries are well placed to respond to traditional BW threats the same cannot be said for newly emerging pathogens. Current approaches, with the exception of the annual influenza vaccine, are not agile enough to respond to a novel biological threat in a time frame capable of limiting the impact of a biological weapon. We urgently need to develop integrated approaches and regulatory frameworks which combine rapid pathogen detection and vaccine target identification with generic platform delivery vehicles capable of mass production. The resulting vaccine should stimulate rapid protection following a single, self administered dose. Such a capability would also need to be coupled with a distribution and dispensing network capable of delivering the treatment to at risk individuals in a realistic time frame. In this presentation I will attempt to summarise some of the approaches currently being pursued to realise this vision.

**EO 02**
**Dengue vaccines - where do we stand and what are the pitfalls?**

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Dengue fever could be life threatening illness which effects more than 390 million people each year. About 96 million cases are with symptoms and about 1% of the cases develop a fatal outcome. There is probably also a high understatement of the disease in the endemic countries. Currently there is no specific treatment available and vector control is not always successful or practicable. The highest number of infections is in Asia and Latin America. Yet there are different dengue vaccine candidates in different clinical development stages. One has already reached the market and is used in some endemic countries. This presentation gives an overview about the different vaccine candidates and their clinical data. The question will be addressed “where do we stand now” and what are the difficulties for the development of a successful dengue vaccine.

EO 03
Update on MVA-BN smallpox vaccine (IM-VANEX/IMVAMUNE): Presentation of phase 3 efficacy data

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Background: Despite its eradication in 1980, Variola virus, the causative agent for smallpox, is still considered a high-priority bioterrorism threat. The risk of re-emergence has prompted governments to stockpile smallpox vaccines as effective countermeasure. MVA-BN(R) vaccine has been developed as a safer alternative to replicating smallpox vaccines (e.g. ACAM2000(R)) which are associated with the potential risk of serious side effects. A recently completed Phase 3 non-inferiority study comparing MVA-BN to ACAM2000, demonstrated indicators of efficacy for MVA-BN.

Methods and Results: 440 subjects were randomly allocated to two groups: Group 1 received two subcutaneous MVA-BN doses followed by one ACAM2000 dose via scarification, given at 4 week intervals. Group 2 received a single ACAM2000 dose. Co primary endpoints were (1) to compare serum neutralizing antibody titers (geometric mean titer [GMT]) as assessed by plaque reduction neutralization test (PRNT) induced by both vaccines at the Peak Visits (Day 42 for Group 1, Day 28 for Group 2) and (2) to assess the attenuation of the ACAM2000 vaccine take after MVA-BN, based on a defined Maximum Lesion Area (MLA). Both co-primary endpoints were met: (1) PRNT GMTs at Peak Visits were significantly higher for Group 1 (153.5) compared to Group 2 (79.3) with a ratio of 1.935 (95% CI: 1.562, 2.397), demonstrating non inferiority compared to ACAM2000. (2) The median MLAs in Group 1 (0.0 mm2) and Group 2 (76.0 mm2) demonstrated a significant relative reduction (area attenuation ratio 97.9% [95% CI: 96.6, 98.3]). Additionally, MVA BN was safe and well tolerated and showed better tolerability than ACAM2000. Vaccinations with ACAM2000 given after MVA-BN priming were better tolerated than ACAM2000 alone.

Conclusions: The Phase III efficacy trial showed MVA-BN to be non-inferior to ACAM2000 and also safer when compared with ACAM2000. Cumulative study data demonstrate that MVA-BN is suitable to protect the general population against smallpox, including those for whom vaccination with replicating vaccines may pose significant safety risks.

BN’s clinical development program is supported by contracts:; DMID N01-AI-30016; DMID N01-AI-40072; HHSO100200700034C; HHSO100201000011C

EO 04
Characterization of CD8 T cell responses recognizing the MERS coronavirus nucleocapsid protein using MVA vector immunization in mice

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Background: In late 2012, a novel coronavirus causing severe lower respiratory disease and deaths in humans was first described. Modified Vaccinia virus Ankara (MVA), a highly attenuated strain of vaccinia virus originating from growth selection on chicken embryo fibroblasts (CEF) serves as one of the most advanced recombinant poxvirus vectors in preclinical research and human clinical trials for developing new vaccines against infectious disease and cancer.

Our objective was to use recombinant MVA compatible with clinical evaluation to express the MERS-CoV candidate antigens spike (S) protein and nucleocapsid (N) protein. The target genes were cloned into MVA vector plasmids and introduced by homologous recombination into the MVA genome. MVA-MERS-S, MVA-MERS-N and MVA-MERS-S/N were genetically stable and replicated efficiently in CEF but not in human HeLa or HaCat cells. S-specific antibodies seem to play a major role in controlling MERS-CoV infection and in mediating vaccine-induced protective immunity. In contrast,
relatively little is known about the role of T cell responses and the antigenic targets in MERS-CoV that are recognized by CD8 T cells. Therefore, the highly conserved nucleocapsid (N) protein is considered a promising target immunogen to elicit MERS-CoV-specific cellular immune responses. Overlapping peptides were used to identify major histocompatibility complex class I-restricted epitopes in mice immunized with MVA-MERS-N or MVA-MERS-S/N. The identification of these epitopes will facilitate studies of immune correlates of protection and the evaluation of vaccine strategies in murine models of MERS-CoV infection.

**EO 05**

Bacteriophages – an antimicrobial option?

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The idea to use phage as treatment option for bacterial infections came up immediately after the discovery of bacteriophage one century ago. As early as in the 1920s, an institute for bacteriophages was founded in Tbilisi, Georgia (former Soviet Union) which is still active. In the following “era of antibiotics”, phage research was focused internationally on the role of these viruses as models in fundamental molecular biology, but their potential significance as therapeutic became underestimated. Under the pressure of global emergence of antibiotic-resistant bacteria and supply shortages for certain antibiotics, the idea of phage therapy was revitalized during the last years; many institutions and commercial companies are currently engaged in this field. There are case histories describing successful phage therapy of single patients, however, the number of controlled clinical studies is still very limited. Besides treatment of antibiotic-resistant bacterial pathogens, phage can be applied as biological control agents for pathogen detection and identification or for food and surface decontamination. Molecular engineering of phage (or phage-derived enzymes) could improve its practical use for these applications. Certainly, detection and treatment approaches of Category A priority pathogens will benefit from these developments.

**EO 06**

Example of a therapeutic approach using intratracheal phage application

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Introduction: Global spread of multidrug resistant (MDR) bacteria is one of the biggest threats in modern medicine. Chronic lung diseases are often complicated by lung and airway infections caused by MDR bacteria, including Acinetobacter baumannii, for which therapeutic options are sparse. Rediscovery of phage therapy may be a solution to the increasing failure of antibiotics. In anticipation of a future clinical trial applying aerosolized lytic phages against gram-negative bacteria in patients with chronic airway infection, this preclinical pilot study aims at determining the efficacy and tolerability of a purified phage preparation.

Methods: Phage Acibel004 [1] was produced as high titer suspension including the depletion of endotoxins using a newly established protocol. Ex vivo, human lung tissue was infected with a MDR A. baumannii strain [2] and treated with phages to determine bacterial growth. Accordingly, infected mice treated 12 h p.i. with specific phages or solvent intratracheally were examined. Clinical parameters, bacterial load in various organs and immune cell influx were determined 24 and 48 hours after infection. Furthermore, lung permeability and cytokine release were quantified and histopathological examination was performed. Moreover, the interaction between phage and A. baumannii was examined using electron microscopy.

Results: Phage application efficiently reduced bacterial burden in ex vivo infected human lung tissue. Mice, treated with phages, showed significantly
reduced bacterial load in bronchoalveolar lavage fluid and lungs as well as a significantly improved clinical outcome. In hematoxylin and eosin stained lung slides of phage treated mice spreading of bacteria to periphery was decreased. Moreover, lung permeability and inflammatory cytokine release were reduced. Immunologically, neither cellular nor humoral unwanted effects of phages were observed. Electron microscopy illustrated the phage-mediated lysis of bacteria.

Conclusion: These data promote the efficiency and safety of purified phage preparation and further support the concept of developing a phage-based therapy against pulmonary *A. baumannii* infections.


**FO 07**

**Engineering bacteriophage with payloads to target multiple bacterial species**

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Bacteriophages (viruses infecting bacteria) are the most abundant biological entity in the biosphere. They are incredibly efficient killers of their bacterial hosts and have been widely used as an alternative to antibiotics in Georgia. With the global antibiotic resistance crisis, there has been increased research into the use of phages as antimicrobials in western medicine. While phages are efficient killers of their bacterial hosts; they can often be specific to certain strains of bacteria. This specificity can both be beneficial and a hindrance. For polymicrobial communities, it may be beneficial to target many different species at the same time. By using synthetic biology approaches, we have been developing methods to engineer the genomes of phages to expand the range of species that a single phage may lyse. We will present data on a model system of *Escherichia coli* and phage T4 that we have engineered to carry additional endolysin genes. Endolysins are produced by most phages at the end of their replication cycle, to lyse their bacterial host and release progeny virions. The endolysins target peptidoglycan and cause lysis from within the cell. Previously endolysins have been used in some commercial products to target Gram-positive bacteria. The lack of a cell wall allows the endolysins to access the peptidoglycan and lyse the cell externally. By incorporating additional endolysin genes, we have been able to engineer T4 so that upon infection of *Escherichia coli* it will release endolysins that will target specific Gram-positive bacteria. To date, we have been able to demonstrate that the lysates resulting from engineered T4 infections can lyse *Staphylococcus* sp. and *Bacillus* sp.

**FO 01**

**Botulinum neurotoxins: New findings on receptor binding and how they translate into innovative detection approaches**

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The exceptional toxicity of botulinum neurotoxins (BoNTs) is mediated by high avidity binding to complex polysialogangliosides and intraluminal segments of synaptic vesicle proteins embedded in the presynaptic membrane. One peculiarity is an exposed hydrophobic loop in the toxins’ cell binding domain Hc, which is located between the ganglioside and protein receptor-binding sites, and that is particularly pronounced in the serotypes BoNT/B, DC, and G sharing synaptotagmin as protein receptor. In the first part of the presentation it is shown that this Hc loop is a critical component of a tripartite receptor recognition complex. Binding to nanodisc-embedded receptors and toxicity were virtually abolished in BoNT mutants lacking residues at the tip of the Hc loop. Surface plasmon resonance experiments revealed that only insertion of the
He loop into the lipid-bilayer enabled high avidity binding of the toxins to membrane-embedded receptors. The results represent a new paradigm of how BoNT/B, DC, and G employ ternary interactions with a protein, gangliosides, and lipids to mediate their extraordinary toxicity (1).

In the second part of the presentation it is shown how these novel findings translate into the development of an innovative animal replacement method for BoNT detection. Finally, future efforts are displayed to evaluate this animal replacement method along with alternative approaches in the EU project EuroBioTox (2).

References:
(2) https://eurobiotox.eu

FO 02
Detection of mycotoxin producers in indoor air via isothermal recombinase polymerase amplification on a chemiluminescence based microarray chip

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Contamination of indoor spaces with mold is a very common problem. Many homes and work spaces have been compromised by moisture at some point, mostly due to water damage. Excess moisture often results in degradation of building material and subsequent mold growth. Exposure rates are especially high where building standards are low, for example in poor countries. The problem of indoor mold causing allergies and infections is long known but hardly dealt with. Also, the fact that indoor molds can produce very harmful mycotoxins is largely undervalued. Mycotoxins are small volatile secondary metabolites produced by fungi. Although meant for microbes, these toxins can cause disease and even death in humans. Examples for dangerous indoor mycotoxins indoors are zearalenone, patulin, ochratoxin and aflatoxin. Especially official institutions dealing with people with sensitive immune systems, such as kindergardens, schools, and hospitals are concerned with this problem. Also when working places are affected, this might have legal consequences. To date the most used methods to test for microorganism contamination in indoor air are culture-based methods which are relatively inaccurate. However, many molds are not culturable. Another method is counting the total number of microbial cells under the microscope which requires a lot of expertise. None of these methods is capable to translate the on-site and real-time situation reliably to analytical data. The aim of this work is to develop a fast and reliable molecular biology-based method to specifically detect mycotoxin producers amongst indoor air contaminants. For this, an isothermal recombinase polymerase amplification (RPA) assay on a chemiluminescence based microarray chip will be developed. This assay is performed on the microarray analysis platform MCR3, which was developed for flow-based chemiluminescence microarrays. In order to specifically detect mycotoxin producers, a bioaerosol sampling method will be conducted that collects molds from air with a high efficiency. From this sample, DNA will be extracted and amplified on a microarray chip by RPA. In contrast to culture based methods, which take 14 days to produce a result on a species level, we will be able to determine if mycotoxin producers are present within just three hours upon sampling. With this assay we aim to develop a screening for highly toxic molds in indoor spaces. If mycotoxin producers can be detected, measures have to be taken instantly.

FO 03
A portable electrochemical biosensor for rapid detection of low molecular weight toxins based on anti-idiotypic antibodies

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Cyano- and mycotoxins, responsible for food- and waterborne diseases, are low molecular weight toxins of high structural diversity with up to 50 toxic congeners within one toxin group. Moreover, they have the potential to be used as biological warfare agents by poisoning food and water resources or by releasing as aerosols in crowded areas. Due to the poisonous nature of these low molecular weight toxins, a rapid and reliable detection system is indispensable to confirm whether an intentional release of the toxin has been occurred to initiate countermeasures. Most of the available detection systems employ a competitive immunoassay format using potentially harmful enzyme-toxin conjugates as competitors. Anti-idiotypic antibodies are a promising alternative to these conjugates by mim-
FO 04
Production and immunological assessment of catalytically inactive Clostridium botulinum holoproteins (ciBoNT HPs)

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Botulism is a neuroparalytic diseases caused by the neurotoxins produced by Clostridium sp. Botulinum neurotoxins (BoNTs) are among the most potent naturally occurring toxins, with estimated human lethal doses on the order of $10^{-9}$ kg$^{-1}$ of body weight. BoNTs are recognized bioterrorism agents that present a distinct threat to U.S. and allied military forces and civilian populations worldwide. There are seven canonical serotypes of BoNT (denoted A-G) and an expanding list of novel new strains and subtypes. The lack of serotype cross protection necessitates the development of polyvalent vaccines for full spectrum prophylaxis.

Currently there are limited preventative medical countermeasures against botulism. The discontinuation of the pentavalent botulinum toxoid (PBT) vaccine (against serotypes A-E) by the Centers for Disease Control and Prevention in 2011 has resulted in the need for a safe and effective prophylactic alternative. Subsequent vaccine efforts have primarily focused on the production of highly purified recombinant protein antigens, representing one or more domains of the botulinum neurotoxin. Recombinant subunit proteins based on the carboxy one-third of the toxin (He domain) has been shown to be safe and effective vaccines against their parental serotypes. A bivalent A/B He vaccine is in Phase II clinical trials in the US.

However, these vaccines are largely untested against dissimilar subtypes. In response to the identification of an ever increasing number of BoNT subtypes with significant amino acid heterogeneity, we have developed full length, catalytically inactive BoNT holoproteins (ciBoNT HPs) in an attempt to elicit broader protective immunity to address these toxin variants.

We have produced ciBoNTs for serotypes A, B, C, D, E, and F. The ciBoNTs have been found to be non-toxic and elicit significant protective immunity against not only their parental toxin, but against dissimilar toxin subtypes as well. The ciBoNTs are highly amenable to polyvalent formulation and a trivalent vaccine against serotypes C, E and F (triCEF) has been developed. The triCEF has been demonstrated to provide significant protection against both individual and combined toxin challenges and has shown outstanding potency when formulated and stored at 4C for up to one year. USAMRIID continues to develop the ciBoNTs as both vaccine candidates and as non-toxic surrogates for use in antibody development.

FO 05
Safe handling of biological toxins – Particular requirements to safety cabinets and isolators

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The handling of biological toxins requires the use of a safe work environment. Appropriate protective measures have to ensure that personnel as well as product protection can effectively be implemented. The current legal situation is complex: As an example in Germany, activities pertaining to isolated toxins are regulated by the German Ordinance on Hazardous Substances (Gefahrstoffverordnung). On the other hand, when activities involve the toxin producing organisms, these activities are also subject to the German Ordinance on Biological Substances (Biostoffverordnung).

In any case, the release of toxins or microorganisms into the air constitutes the main exposure route. With regard to occupational safety and health, inhalation of particles or aerosols inadvertently generated during work or as the result of a spill must be prevented.

Safety cabinets and isolators are considered to be the most effective technical measures to minimise this risk. They should provide a secure containment, which prevents airborne particles from escaping or entering the working area. Despite their widespread use, there are so far no regulations which define the particular requirements for these devices when used as a barrier system against biological toxins.

In Germany, since 2017, the standard DIN 12980 defines the design and testing procedures of safety cabinets and isolators, which must be used for the handling of substances classified as carcinogenic, mutagenic, or toxic for reproduction (CMR substances). Moreover, in the related standard DIN EN 12469 basic requirements for microbiological safety cabinets (classes I to III) are specified.

In line with both standards and in view of our own long-term experience, the special requirements for the safe handling of biological toxins should be identified and discussed. The ongoing efforts at federal and European level in connection with the amendment of the DIN EN 12469 make these considerations more topical than ever.

Biological toxins are known as causative agents of food poisoning outbreaks, but some of them also have a history as warfare agents and thus could be used in a bioterrorism context (e.g. Clostridium botulinum neurotoxins (BoNTs), ricin, Staphylococcal enterotoxins (SEs)). Recent events in Cologne in June 2018 have shown that the attempted production and release of ricin is a realistic threat scenario. Results from previous proficiency tests performed in the EU project EQuATox (2012-2014) provided a sound basis for quality assurance measures and highlighted the potential for further technical improvement (1, 2). EuroBioTox is an ongoing Horizon 2020 project under the EU Framework Programme for Research and Innovation with 13 consortium partners and 50 network partners from 23 countries including the health, food, military and verification sectors. The project aims at establishing a Pan-European network of competence for the detection and analysis of biological toxins of potential bioterrorism threat (3).

Using current best practice, the EuroBioTox core members will develop and validate improved analytical tools, reagents and standard operating procedures based on realistic incident scenarios. Alternative in vitro tests for the current but ethically unacceptable animal test for botulinum neurotoxin will also be evaluated. Certified Reference Materials (CRM) for the threat biotoxins will be developed and, by establishing a European repository, will be made available to the EuroBioTox network and other competent and authorized end-users. Training courses at basic and advanced levels will be offered to the EuroBioTox network partners, followed by a series of proficiency tests to disseminate best practice methods across

FO 06
European Programme for the Establishment of Validated Procedures for the Detection and Identification of Biological Toxins (EuroBioTox)

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GO 01
Antivirals & antibiotics development
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Drug discovery is a time-consuming, expensive process and is often a very frustrating experience for the researchers involved. In addition, these initiatives are often influenced by business-related considerations that could determine the key involvement of private companies in the discovery process. This is especially relevant in the context of the development of novel antibiotics and, to some extent, new antiviral agents. For this reason, researchers in these fields are exploring a variety of strategies and methodologies in order to reduce the time and costs of drug discovery. In this talk, some examples of these approaches will be discussed, including, but not limited to, computer-aided drug design applications, and drug repurposing.

GO 02
In silico design and synthesis of novel antiviral nucleosides and their prodrugs as potential treatments for arbovirus infections
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Vector-borne emerging diseases such as Chikungunya and Zika infections currently represent global concerns, due to their recent outbreaks in the Americas, Europe, and the US. Chikungunya virus (CHIKV) is an Arbovirus associated with an acute pathology characterised by fever, rash and arthralgia, a condition which is often severe and may persist for several months or become chronic in the 10% of infected individuals. Zika virus (ZIKV) is a Flavivirus also transmitted to humans by mosquitoes, responsible for an acute febrile illness associated with severe neurological complications, such as the Guillain-Barré syndrome in adults, and microcephaly and neurological disorders in newborns to women infected during pregnancy. Currently, no therapeutic options are available to treat these two viral diseases. Among the viral non-structural proteins responsible for virus replication, the polymerase is a promising target for antiviral drug design, as its inhibition with nucleoside analogues is one of the most successful strategies in antiviral drug discovery for several other viruses. Using computational approaches, a homology model was built for CHIKV nsP4 polymerase, using the Norwalk virus polymerase as template. The resulting structure was optimised by including the nucleic acid components of the elongation complex, the RNA template strand, the RNA growing strand and the incoming nucleoside triphosphate. In a similar fashion, a model for the active complex of ZIKV NS5 polymerase with the nucleic acid elements was built using the HCV polymerase complex structure as template. The two models were validated by docking known nucleoside inhibitors of the two viruses, in their triphosphate forms, in the NTP binding site, and used to screen in silico a virtual library of novel potential nucleoside inhibitors. These calculations guided the selection of new nucleosides with a high predicted affinity for the two polymerases, and the best hits were chosen for chemical synthesis, along with their phosphoramidate prodrugs. The rational design and synthesis of these novel compounds will be discussed.

GO 03
Biological evaluation of novel small-molecule antiviral agents versus tick-borne encephalitis virus
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Tick Borne Encephalitis Virus (TBEV) is a flavivirus causing a flu-like illness and meningoencephalitis in the human host. TBEV is transmitted by Ixodes ticks and endemic in temperate climate zones suitable for the vector, mainly in Europe and Asia, but not in the Americas. A number of approved inactivated TBEV vaccines are on the market, but so far, there are no TBEV specific therapeutics. A number of compounds previously found active versus flaviviruses and non-toxic in human HUH7 hepatoma cells, were tested versus TBEV in human cell lines where TBEV causes cytopathogenic effects (CPE). Several compounds with IC50 in the µM range and minimal toxicity were identified. The current lead compound D12 inhibits TBEV with an IC50 of 3.5µM and a selective index of 52 in HUH7 cells. Further biological evaluation of these compounds in different cell lines, including central nervous system cells, and different assay methods is ongoing and will be reported. This work provides the foundation for further investigation of promising novel structures as antiviral agents against TBE virus.

GO 04
New agents targeting ZIKV protease or methyl-transferases

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Flaviviruses are positive single stranded RNA viruses belonging to the Flaviviridae family. Most of them, including dengue virus (DENV) and Zika virus (ZIKV) are mainly transmitted by Aedes mosquitoes. DENV and ZIKV viruses, once found only in some areas, are now widespread and have shown the potential to become epidemic and endemic, as proven by the DENV expansion and the outbreak of Zika fever. Dengue and Zika infections show similar symptoms, such as fever, joint pain, rash, and may progress to more serious complications, haemorrhagic fever and shock syndrome for DENV, and Guillain-Barré syndrome and microencephaly in new born for ZIKV. Despite the recent live-attenuated DENV vaccine, there are no effective drugs to treat DENV and ZIKV infected people. We performed virtual screening (VS) studies at the allosteric site of the DENV NS2B/NS3 protease to design and synthesize new DENV NS3 protease inhibitors. Indole compounds 1 and 2 showed strong inhibition against the DENV protease with EC\textsubscript{50} of 6.71 ± 0.20 µM and 7.92 ± 0.62 µM in the cell-based DENV protease assay. In the enzymatic assay, 1 and 2 specifically suppressed DENV protease activity with EC\textsubscript{50} of 4.72 ± 0.3 µM and 6.90 ± 0.11 µM, respectively (1). Homology modeling studies of the NS3 protease showed high level of homology between the DENV and ZIKV protease in the catalytic pocket. These findings prompted us to evaluate compounds 1 and 2 and some analogues (i.e. compound 3) as inhibitors of ZIKV protease. Compounds 1 and 3 exhibited EC\textsubscript{50} of 10.9 and 6.7 µM, respectively, in a ZIKV infectious system in HepG2 cells. In a kinetic binding assay of ZIKV protease, compounds 1 and 3 showed a non-competitive-inhibition mechanism with Ki values of 41 and 276 µM, respectively. A series of analogues of 1 and 3 were evaluated in HepG2 cells against ZIKV strain Uganda. Seven compounds had efficacy versus Zika Virus in HepG2 cells and worked up to 10 times better than Ribavirin; none of the compounds tested were toxic. These findings show that these compounds have potential for the development as drugs to treat ZIKV infection.

References:

GO 05
Biological evaluation of natural extracts versus poxviruses

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Vaccinia Virus (Poxvirus family) is closely related to other viruses in the Orthopoxvirus genus, e.g. Variola Virus, Monkeypox and Cowpox [1], and can be used as a surrogate model of Orthopoxvirus infection in antiviral studies at BSL2. Vaccinia WR is associated with Postvaccinial Encephalitis, a rare but severe complication of Smallpox vaccination with replicating viruses [2]. Encephalitis was regularly observed in Smallpox cases. In this project we have used fully replication competent Vaccinia Virus strain WR to test the antiviral
activity of natural plant extracts prepared in the laboratory of Les Baillie, School of Pharmacy and Pharmaceutical Sciences in Cardiff.

A luciferase-expressing vaccinia virus WR (v3-LUC240) was used to both investigate the capability of the substances to prevent the early stage of infection (virus entry; at 4 hours p.i.- reporter activity) and the later stage of infection (at 3 days p.i.- viability assay; at 5 days p.i.- plaque reduction assay). The extracts were screened at a single concentration; the activity and toxicity spectra of effective extracts were further characterized. The clinically approved drug Cidofovir (CDV) and the experimental autophagy inhibitor cf2642 were used as inhibition controls.

Organotypic cell lines A549 (human alveolar epithelial cell; lung), HUH-7 (human hepatoma; liver), and DBTRG (human glioblastoma; brain) were used.

From preliminary data, we have found that *Taraxacum officinale* (Dandelion) extract fraction 9 (BB4-D9) tested at 6.4µg/mL had an efficacy comparable to CDV at 10µM in A549. Further subfractions of BB4-D9 are to be tested to identify the active component of *Taraxacum officinale* plant extract.

**GO 06**

**Evaluation of novel inhibitors against the macrophage infectivity potentiator in biowarfare agents**

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The Macrophage infectivity potentiator (Mip) protein is a virulence factor encoded by intracellular pathogens. *Burkholderia pseudomallei*, recognized as a CDC Tier 1 select agent, is the causative agent of melioidosis a disease endemic in South East Asia and Northern Australia. There is currently no vaccine available against *B. pseudomallei* and the organism is resistant to a range of antibiotics, therefore identification of new drug targets is essential. The Mip protein in *B. pseudomallei* exhibits virulence-associated peptidyl-prolyl isomerase (PPIase) activity, inhibition of which potentially represents a novel target for antimicrobial therapies to this organism. A group of piperolic acid derivatives have been shown to inhibit Mip from *L. pneumophila*. Using a protease coupled PPIase assay, we have demonstrated that a selection of these piperolic acid derived compounds also have inhibitory properties against the recombinant *B. pseudomallei* Mip. Importantly, these inhibitors reduce the cytotoxic effects of *B. pseudomallei* on macrophages, therefore presenting as novel therapeutics to this agent.
Smallpox is designated a material threat to the US, and until TPOXX approval, antiviral treatment options represented a serious unmet need. SIGA Technologies has developed TPOXX for the treatment of smallpox, and has already supplied two million treatment courses to the US Strategic National Stockpile. The US FDA Animal Rule guided TPOXX drug development, as clinical trials are impossible to conduct due to eradication of naturally occurring disease and ethical concerns. TPOXX efficacy was demonstrated in pivotal animal studies conducted in FDA-accepted non-human primate (NHP) and rabbit models for human smallpox. The NHP is the more conservative of the two models as higher drug exposures are required for maximal efficacy. SIGA conducted a Phase 3 randomized, placebo-controlled study in which subjects were allocated to receive 600 mg TPOXX (359 subjects) or placebo (91 subjects) twice daily for 14 days. Data from the Phase 3 study demonstrated that drug exposures in humans exceeded those required for efficacy in NHPs, providing a reasonable expectation of efficacy in humans. There were no drug related SAEs, and no safety signals were identified. Most reported adverse events were mild and all drug related events resolved without sequelae.

In May 2018, the FDA Antimicrobial Drug Advisory Committee voted 17-0 in favor of TPOXX benefit versus risk, and in August 2018, FDA approved TPOXX for the treatment of smallpox. Post-marketing requirements include conducting a field study to verify and describe the drug’s clinical benefit when feasible and ethical, and labeling to indicate approval is based on efficacy demonstrated in animals alone. Post-marketing commitments requested by FDA include environmental impact, elemental impurities, drug-drug interaction, and expanded in vitro drug activity studies. FDA also requested that SIGA conduct a study in subjects with body weight greater than 120 kilograms to determine if a change in dosing is needed in these subjects. These studies do not affect drug approval but may result in label refinements. SIGA has now transitioned to post-marketing development of TPOXX.

Smallpox vaccines were used effectively for eradication of smallpox, but also have been associated with adverse events, some severe. The 21st century population has an increased number of individuals with conditions (HIV, cancer treatment, etc.) which make them more susceptible to these severe adverse events. Development of vaccines protective for these populations is important to protect global health in the event of smallpox reemergence (natural or malicious). Mutiple less-reactogenic vaccines have been produced and tested against *Vaccinia virus*, but were never tested for efficacy against smallpox. Variola major virus and Variola minor virus (Alästrim) are the causative agents of smallpox and are select agents subject to the select agent regulations (42 CFR Part 73). *Variola virus* (VARV) neutralization in vitro is an informative surrogate measure of smallpox vaccine efficacy, however the plaque reduction neutralization test (PRNT) has demonstrated neutralization of surrogate orthopoxviruses does not accurately predict the neutralization potential of the sera against VARV. As serum neutralization is known to vary dependent upon the target virus (even when closely related viruses are used), a VARV PRNT was developed at CDC. The parameters of the assay were standardized and reproducibility determined. Coefficients of variation between operators and between days were 4.31% and 5.49% (Log10 titer) respectively. The assay will be of importance to evaluate sera from a clinical trial which demonstrated Bavarian Nordic’s IMVAMUNE (a next-generation, non-replicating smallpox vaccine) is non-inferior to the currently licensed, replicating smallpox vaccine (ACAM2000). The data on VARV neutralization will provide additional data supporting the IMVAMUNE regulatory review for the Food and Drug Administration. If approved, this will be the first next-generation smallpox vaccine to gain licensure for the United States.
E3L and F1L gene functions modulate the protective capacity of Modified Vaccinia virus Ankara immunization in murine model of human smallpox

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The highly attenuated Modified Vaccinia virus Ankara (MVA) lacks most of the known vaccinia virus (VACV) virulence and immune evasion genes. Today MVA can serve as a safety-tested next-generation smallpox vaccine. Yet, we still need to learn about regulatory gene functions preserved in the MVA genome, such as the apoptosis inhibitor genes F1L and E3L. Here, we tested MVA vaccine preparations on the basis of the deletion mutant viruses MVAdF1L and MVAdE3L for efficacy against ectromelia virus (ECTV) challenge infections in mice. In non-permissive human tissue culture the MVA deletion mutant viruses produced reduced levels of the VACV envelope antigen B5. Upon mousepox challenge at three weeks after vaccination, MVAdF1L and MVAdE3L exhibited reduced protective capacity in comparison to wildtype MVA. Surprisingly, however, all vaccines demonstrated equally protective against a lethal ECTV infection at two days after vaccination. Accordingly, the deletion mutant MVA vaccines induced high levels virus-specific CD8+ T cells previously shown to be essential for rapidly protective MVA vaccination. These results suggest that inactivation of the anti-apoptotic genes F1L or E3L modulates the protective capacity of MVA vaccination most likely through the induction of distinct orthopoxvirus specific immunity in the absence of these viral regulatory proteins.

Recombinant VIG: Development of a potential antibody-based therapeutic for smallpox vaccine adverse reactions

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Smallpox is considered a major bio-threat agent and vaccination is the only viable protection against disease spread. Smallpox vaccine consists of live vaccinia virus (VACV) which was used worldwide to eradicate this disease. Currently the vaccine is in use to vaccinate laboratory workers while stockpiled to prepare for possible smallpox reemergence. To manage the rare yet severe adverse reactions to this vaccine, an FDA approved plasma-derived vaccinia immune globulins (VIG) is isolated from vaccinated donors. However, VIG has limited potency, is in short supply and has batch-to-batch variations in activity. Thus, we incorporated our in-depth knowledge of immunization methodologies to elicit high affinity antibodies in vivo, together with efficient screening methods using phage-display libraries in order to isolate anti-VACV antibodies that will serve as a recombinant alternative to VIG. To this end, two non-human primates were vaccinated with live VACV to ensure the elicitation of diverse high affinity antibodies. By using a comprehensive set of primers, immune scFv phage-displayed libraries were constructed and panned against the virus or against purified recombinant surface proteins. Antibodies directed against the viral proteins H3, A33 and B5 (representing the two infectious virion forms) were isolated and reformatted into full-length chimeric IgG format. These antibodies exhibit very high affinity toward VACV and were found to possess excellent neutralization activity, resulting in 1000-fold improvement over the commercially available VIG. Preliminary data also suggest that these antibodies can be effective in vivo and protect mice from lethal VACV infection. We believe that a combination of these novel antibodies can be used for the development of a recombinant, highly-effective therapeutic for the treatment of VACV vaccine-associated severe adverse reactions.

Use of next generation sequencing to study two cowpox virus outbreaks

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The first outbreak accounted for about 40 cases of human cowpox which were reported from Germany and France, 2008 and 2011. Infections had been acquired via close contact to infected, young pet rats. Sequencing of the hemagglutinin gene of var-
ious cowpox virus isolates resulted in an identical and unique sequence in each case and a common source was assumed. In a second outbreak in 2015 in a small animal clinic in Germany, four out of five hospitalized cats showed identical hemagglutinin sequences and thus, a hospital-acquired transmission was assumed. Next generation sequencing (NGS) was used to further investigate both outbreaks. Homogenates of lesion material from rats and humans were cultivated in cell culture. Sixteen genomes (4 virus isolates, 9 CPXVs from our strain collections and 3 from DNA of paraffin-embedded lesion materials) were determined by NGS. For phylogenetic analyses a MAFFT-alignment was generated. A distance matrix based on concatenated SNPs was calculated and plotted as dendrogram using Unweighted Pair Group Method with Arithmetic mean (UPGMA) for visualization. Alignment of about 200,000 nucleotides of 8 virus isolates associated with the pet rat outbreak revealed complete identity of 6 genomes, the remainder 2 genomes differed in as little as 3 SNPs. When comparing this dataset with four already published CPXV genomes associated with the pet rat outbreak, again a maximum difference of 3 SNPs was found. Therefore, we conclude that the pet rat-associated outbreak which lasted from 2008 till 2011 was indeed caused by a single strain which has maintained an extremely high level of clonality over 4 years. Aligning genomic sequences from 4 cases of feline cowpox revealed 3 identical sequences and one sequence which differed in 65 nucleotides. Although identical hemagglutinin sequences had been obtained from four hospitalized cats, genomic sequencing proved that a hospital-acquired transmission had occurred in only three cats.; Determining the sequence of the hemagglutinin gene is not sufficient to conduct molecular trace-back analyses. Instead, whole genome sequencing is the method of choice which can even be applied to paraffin-embedded specimens.

**HO 07**

**MPXV in travellers returning from Nigeria, UK 2018**

V Graham  

In early September 2018, two cases of monkeypox were reported in the United Kingdom. The cases were epidemiologically unconnected and had recently travelled to the UK from Nigeria, where monkeypox virus is currently circulating. A third case of nosocomial transmission to a healthcare worker was also reported. A summary of the situation will be presented.

**Recent Advances in Targeted and Open View Diagnostic Procedures**

**IO 01**

**Diagnostic electron microscopy of pathogens in emergency situations**

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Globalisation, interlinked human communities with a high mobility at different levels path biological threats new ways, which can reach almost every region on our planet in short time. Preparedness to fight biological threats, like the transmission of highly infectious diseases or bioterrorism, must comprise several aspects, including the rapid search for pathogens. Diagnostic electron microscopy (DEM) of pathogens is a rather old method to detect and to characterise pathogens, but, because of its speed and generic catch-all capability, it is a valuable part of the diagnostic panel in emergency cases, such as outbreaks or putative bioterrorist attacks [1]. The presentation will describe the principles of the pathogen detection and recognition by DEM and why it is comparably quick to perform. Negative staining electron microscopy (EM) is the main method to prepare samples for DEM, because it is simple and quickly (within 15 min) performed [2]. However, thin sectioning EM of samples, which are too thick for negative staining EM (e.g. tissue biopsies), can also be performed at a very short time (i.e. within 2 h) [2]. Biosafety in the lab is an important aspect in dealing with putatively highly infectious samples, but can be addressed with reasonable means [3]. Advantages and disadvantages of DEM will be discussed and research is presented on how disadvantages can be possibly circumvented. An outlook on further developments, like automa-
tion and easier to operate instrumentation, will be provided. Finally, a couple of examples for the application of DEM in the past with an outlook to the future should demonstrate that DEM is a beneficial part of pathogen diagnostics in emergency situations.

References:

IO 02
De-novo detection of viral pathogens with adaptive diagnostics and integrated data analysis approaches

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Novel and (re)emerging viruses cause frequent threats to both human and animal health. Diagnostic sequencing by unbiased next-generation sequencing is the key method for the identification of new pathogens. One major barrier for the use of this method in day-to-day diagnostics is often the lack of standardized workflows and data analysis tools in a user-friendly environment. The interdisciplinairy project “DetektiVir” aims at closing this gap by deploying a new workflow which combines molecular nucleic acid-based virus detection by metagenomic sequencing with ad-hoc development of customized serological diagnostics, and integrates data in dynamic database applications.

Central part of the new workflow is a novel diagnostic data hub application that combines raw sequence reads and metadata with the results from taxonomic classification software in a database environment. This core system offers flexible data interfaces and software algorithms in a user-friendly environment. The application was evaluated with samples from diseased animals infected with unidentified pathogens. Analyses comprised next-generation sequencing, data analysis and integration of data in the data hub. In case of sufficient data, full viral genomes were assembled and phylogenetically classified. Confirmation of the findings by RT-qPCR as well as attempts for virus isolation and electron microscopy were performed.

Applying the workflow, we were able to discover novel viruses. We identified e.g. a novel picornavirus, tentatively named ovine picornavirus, from 2-3 week old lambs in the UK, suffering with polioencephalomyelitis and ganglionitis. We also detected and classified a novel paramyxovirus, which was isolated from a diseased grizzled giant squirrel of Sri Lanka and is therefore preliminarily designated giant squirrel respirovirus. Interestingly, this novel virus has an overall pairwise sequence identity of 71% with known murine respiroviruses and 68% with human respiroviruses.

The identification of novel viruses proves the strength and suitability of the new workflow. By integrating the gathered information, including metadata, into the dynamic database the growing information will pave the way for earlier identification of associated outbreaks of a potential novel pathogen.

IO 03
Automation and workflow optimization in a molecular lab – adaptation ability to a military biothreat facility?

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The routine workflow in a molecular diagnostic laboratory is dominated by an expansive spectrum of methods and protocols to identify pathogenic targets. Additionally, different clinical specimen types and from time to time rising sample numbers, e.g. during an outbreak scenario, pose a challenge to the capability of the lab. Workflow optimization and automation strategies are therefore crucial to enable high throughput investigation with a maximum of efficiency and information content. In our molecular laboratory we have established an optimized combination of sample management, automated extraction and detection protocols, multi-target amplification, bidirectional order entry and reporting setting followed by a systematic digital archiving procedure. Different automated instrument platforms have been connected to a unique flow of work.

Here we describe our workflow starting with the received sample. The sample management organizes the control and LIS registration of the barcode labeled tubes. Piercable caps enable a contamination free access to the sample and a transport solution inactivates bacteria and viruses to minimize the risk of transmission. Using extraction instruments connected to the LIS we can theoretically proceed up to 1000 samples in a 12 hours working day. In a real world scenario we usually handle 250-500 samples per day with increasing numbers during the flu season or Noro-virus outbreaks. After NA-extraction the amplification and pathogen
detection run in multiple pathways depending on the requested order. The LIS is bidirectionally connected to the TC devices. The generic amplification protocols combine multi-target detection of different pathogens as well as highly specific single-target assays. After QC-inspection the results are transferred directly into the LIS for validation and reporting. The sample tubes are archived including an electronic place marker for subsequent investigation (e.g. resistance testing, NGS typing, etc.). The result data can be further used for bioinformatical and biostatistical evaluation for QC and outbreak surveillance. This workflow is adapted to the special laboratory requirements and is essential for a quality managed process. It is not only suitable to help avoid sample contamination and inadvertent mix-ups, but it also enables sample traceability in both civil and military laboratory environments. By integrating the already existing highly sensitive and specific molecular methods into a combined work process and by implementing new technologies such as Next Generation Sequencing, automation is already a fundamental principle in laboratory processes but is also indispensable for conducting outbreak investigations.

IO 04

Unbiased identification of highly pathogenic bacteria from blood culture flasks by analyzing the peptidome

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Bloodstream infections (BSI) are a common cause of disease, which can be caused by a wide variety beforehand unknown microorganisms, including highly pathogenic bacteria. Currently, the process to identify a causative pathogen of a BSI can be, depending on the type of pathogen, time-consuming and delayed due to the lack of confidence in the initial characterization results.

To strengthen our biodefence capabilities and to improve our preparedness against exposure to highly pathogenic bacteria a safe, unbiased and highly accurate identification method is developed. The liquid chromatography-tandem mass spectrometry-based method is able to identify highly pathogenic bacteria, their near-neighbors and bacteria that are a common cause of BSI direct from positive blood culture flasks. The developed online tool Peptide-Based Microbe Detection Engine (http://proteome2pathogen.com/app/) analysis the peptidome using a two-step workflow: a genus-level search followed by a species level search.

This method is successfully used for identification from blood culture flasks for Bacillus anTHRACIS, Brucella abortus, Brucella melitensis, Brucella suis, Burkholderia pseudomallei, Burkholderia mallei, Francisella tularensis, Yersinia pestis, related species and other bacteria that can cause BSIs. The developed LC-MS/MS method is a safe and rapid method which enables the identification of bacteria, directly from positive blood culture flasks without prior knowledge.

IO 05

Development of a comprehensive MALDI-TOF MS spectral database for identification of Brucella spp. at species level by statistical learning algorithms

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Brucellae are highly infectious bacterial pathogens classified as Category B security-sensitive biological agents by the US Center for Disease Control and Prevention. Brucellosis, the associated zoonosis, is reported with more than 500,000 human cases worldwide every year. In addition, infection of livestock results in severe economic losses in endemic countries. Current procedures for the microbiological typing of Brucellae are expensive, time-consuming and restricted to biosafety level 3 containment laboratories.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an emerging routine diagnostic tool for rapid bacterial identification that relies on the presence of reference spectra in the instrument-specific typing databases. The genus Brucella with its twelve species at present is still not sufficiently covered by approved in-vitro diagnostic databases and ill-defined by research-use-only or security-relevant extension libraries. Consequently, the current MS-based diagnostic methods allow for identification of the genus Brucella but discrimination of Brucella species may fail.

We analyzed more than 600 Brucella isolates following a rapid sample inactivation and preparation protocol covering all known Brucella spp. including rare isolates from novel animal hosts. The collection of approximately 16,000 raw spectra was preprocessed in the free, open-source statistical computing environment R, facilitating parameter optimization and subsequently, the analysis by advanced statistical learning algorithms. Thus, linear discriminant analysis identified species-specific
biomarkers and ranked them by predictive potential while tree ensemble models were tested for their classification accuracy, sensitivity and specificity by cross validation. Moreover, a challenge with publicly available Brucella MS spectra allowed the assessment of classification robustness with respect to different laboratories, instruments and spectral qualities as well as inactivation and sample preparation protocols.

By expanding the reference database combined with the application of modern computational statistics we significantly improved MS-based discrimination of Brucellae at species level.

**IO 06**

**Synthesis of a new antigen for the detection of Tula orthohantavirus in central Germany**

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Zoonotic pathogens that can cause severe disease in humans are on the rise worldwide. Rodents harbor several pathogens relevant for public health including species-specific pathogens in terms of rodent host species such as orthohantaviruses. The common vole (Microtus arvalis) harbors a number of rodent-borne pathogens including Tula orthohantavirus (TULV) - a pathogen specific to common voles and field voles (M. agrestis). This study aimed to determine the prevalence of TULV-specific RNA and antibodies in common voles. Small mammals were snap trapped during 2017 and 2018 in the 'Thüringer Becken', a central German region known for intensive large-scale agriculture where outbreaks of the common vole occur about 2-5 years. Previous studies detected TULV in common voles in this area. We sampled 705 voles and analyzed lung samples for TULV using a standard S-segment-specific RT-PCR with subsequent sequence determination, as well as analyzing the presence of TULV-reactive antibodies by ELISA with a newly generated recombinant antigen. This new antigen was synthesized in yeast (Saccharomyces cerevisiae) based on a nucleocapsid protein encoded sequence of a common vole-associated TULV strain from Thuringia.

First results show that the detection of antibodies against TULV in common voles is possible with the new antigen. Ongoing investigations will have to show potential antigenic differences between nucleocapsid proteins from TULV strain Thuringia, Germany and Moravia, Czech Republic.

**IO 07**

**Novel minimally invasive diagnostic devices to screen infectious diseases**

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The U.S. Department of Defense is pursuing the development of low cost, highly specific, and minimally invasive Clinical Laboratory Improvement Amendments (CLIA) waived lateral flow assays (LFA) for the diagnosis of infections caused by biological warfare agents (BWAs). LFAs are perfect diagnostic candidates for use in austere environments due to their ease of use, familiar format, and reasonable sensitivity. The Defense Threat Reduction Agency (DTRA) is contracting with industry to develop Food and Drug Administration cleared LFAs for use as a CLIA waived in vitro diagnostic device. The goal is to provide field-forward deployable point-of-need diagnostics to warfighters that may become infected with BWAs either naturally or intentionally. Discovering unique secreted pathogen-specific targets in urine or capillary blood (finger prick) represents the least invasive samples.

DTRA is currently pursuing the development of LFAs for two pathogens: Burkholderia pseudomallei (causative agent for melioidosis) and Yersinia pestis (causative agent for plague). In the case of Burkholderia pseudomallei infections, where free bacteria are rarely present in blood, capsular polysaccharide (CPS) proteins secreted by the pathogen are expelled from the body through the urinary system. This makes urine the ideal clinical sample for diagnostic testing using the LFA. Alternatively, Yersinia pestis secreted proteins can be found in capillary blood.

Preliminary data for the Burkholderia pseudomallei LFA demonstrates that it can detect CPS in the picograms levels. DTRA is conducting preclinical validation studies at the U.S. Army Medical Research Institute for Infectious Diseases and at hospitals in melioidosis endemic regions in Thailand and northern Australia. Research and development of a two target Yersinia pestis LFA is currently ongoing. DTRA plans on collaborating with the Center for Disease Control and Prevention and
hospitals in plague endemic regions in Madagascar and Uganda for pre-clinical validation studies. The development and use of these LFAs will greatly benefit warfighters as well as point of care clinicians in austere environments.

Focus Session

Secrets Hidden in the Depth of Genomes

Chairs: H.C. Scholz (DEU) and G. Vergnaud (FRA)

JO 01

The genetic history of the plague: From the Stone Age to the 18th century

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High throughput DNA sequencing has revolutionized the field of archaeogenetics in the past decade, providing a better understanding of human genetic history, past population dynamics and host pathogen interactions through time. Targeted DNA capture approaches have allowed reconstructing complete ancient bacterial genomes providing direct insights into the evolution and origin of some of the most infamous bacterial pathogens known to humans such as *Yersinia pestis*, *Mycobacterium tuberculosis* or *Mycobacterium leprae*. Here we discuss the potential of ancient pathogen genomics using *Yersinia pestis* as a model organism. Phylogenetic comparisons of modern and ancient *Y.pestis* strains spanning over 5000 years of human history from the Stone Age to modern times are discussed. They provide direct evidence for the timing and emergence of major virulence factors essential for the transmission of bacteria by fleas. We furthermore present the oldest reconstructed genomes of *Y.pestis* that are fully capable of causing the bubonic form of plague from the Eastern European Bronze Age. Suggesting that the emergence of this form of the disease happened more than 1000 years earlier than previously suggested. Temporal studies of pathogens might thus throw new light on the origin of human diseases and potentially allow predicting and preventing further transmissions and disseminations in the future.

JO 02

*Bacillus anthracis* evolutionary history: Taking advantage of polytomy and human history to propose dating points

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Bioforensics is an important topic within biodefense. A demonstrated capacity to link a biothreat event to a source (source attribution) has the potential to lower the attractiveness of biological weapons. Source attribution by genetic analysis of the pathogen relies upon the interpretation of genetic differences observed between different representatives to propose a dating of most recent common ancestors (MRCA). In a real-case situation, one of the datasets will be associated with an alleged bioattack, and the other will be a reference database of sequenced genomes. Single nucleotide polymorphism (SNPs) will constitute the primary set of genetic variations to investigate, because they are robust, especially when dealing with potentially degraded DNA samples and associated short sequence reads. Comparing sequence data sets will result into lists of SNPs defining “distances”. The dating of *Bacillus anthracis*, which is considered as the main potential biothreat agent, is particularly challenging due to its dormant, sporulated state and resulting absence of a regular molecular clock. For biodefense purposes, the population structure of *B. anthracis* has been characterized in detail during the past twenty years. Owing to its strictly clonal evolution, lineages could be conveniently defined by using carefully selected SNPs, called canonical or canSNPs. More recently, insights from systematic whole genome sequencing is beginning to provide tentative dating points. The talk will discuss the remarkable A.Br.011/009 polytomy, i.e. the recently described star-like radiation pattern of evolution with six branches of strains assigned to canSNP A.Br.011/009. Sequence reads archives and closed genomes datasets from seven hundred *B. anthracis* strains were recovered from public databases. SNPs were identified by mapping each genome on the Ames strain reference genome sequence. Fifty-four datasets could be assigned to canSNP A.Br.011/009 and eighty-six more to the A.Br.WNA sublineage predominant in North America. A total of 2038 SNPs was identified within A.Br.011/009. Sequence reads archives and closed genomes datasets from seven hundred *B. anthracis* strains were recovered from public databases. SNPs were identified by mapping each genome on the Ames strain reference genome sequence. Fifty-four datasets could be assigned to canSNP A.Br.011/009 and eighty-six more to the A.Br.WNA sublineage predominant in North America. A total of 2038 SNPs was identified within A.Br.011/009. Sequence reads archives and closed genomes datasets from seven hundred *B. anthracis* strains were recovered from public databases. SNPs were identified by mapping each genome on the Ames strain reference genome sequence. Fifty-four datasets could be assigned to canSNP A.Br.011/009 and eighty-six more to the A.Br.WNA sublineage predominant in North America. A total of 2038 SNPs was identified within A.Br.011/009.
from Canada and West Africa. Interpretation of the topology of the polytomy combined with historical events allows to propose a precise dating for its emergence in spite of the very different evolution rates among lineages.

**JO 03**

**Genotyping of Bacillus anthracis strains isolated from cattle in Finland**

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Anthrax is caused by the bacterium *Bacillus anthracis* and is nowadays rare in Finland. Since the 1970’s, only two small outbreaks have been detected in cattle in the years 1988 and 2004 and one additional sporadic infection occurred in 2008. Isolates from 1988 and 2008 were named *B. anthracis* strains HKI4363/88 and BA2968, respectively. Here, both strains were subjected to PCR-based genotyping and whole genome sequencing (WGS).

The genomic DNA of these two strains was analysed by multilocus variable-number-of tandem repeats analysis MLVA-31 and by canonical single nucleotide polymorphism (canSNP) typing scheme in which mismatch amplification mutation assays (Melt-MAMA) for 12 canSNP groups were performed. The genome sequences produced by MiSeq (Illumina) were subjected to chromosomal single nucleotide polymorphism (SNP) analysis using the Parsnp tool and an ad-hoc core genome MLST analysis (cgMLST) using Ridom SeqSphere+ software. The SNP results of *B. anthracis* strains HKI4363/88 and BA2968 and the allelic differences obtained from cgMLST were compared and visualized as a minimum spanning tree (MST).

Strains HKI4363/88 and BA2968 showed differences in 23 out of 31 MLVA loci and 719 allelic differences to each other in the MST as evident from the gene-by-gene analysis in which 4952 core genome genes were compared. Phylogenetic comparison of the two strains by SNP analysis grouped these organisms within their relatives of the minor canonical A-branch canSNP-group A.Br.003/004 (A.Br.V770) or canonical B-branch B.Br.001/002, respectively. Strain HKI4363/88 clustered relatively closely with other members of the A.Br.003/004 lineage from Europe, South Africa, and South America. In contrast, strain BA2968 clearly constituted a new sublineage within B.Br.001/002 with its closest relative being HY001 from South Korea.

About 90% of the reported global outbreaks are caused by *B. anthracis* strains belonging to the A-clade. These results suggest that Finland harbors both unique and more widely distributed clades of *B. anthracis*. We suspect that members of the common clades such as strains HKI4363/88 have been introduced only recently by anthropogenic activities involving importation of contaminated animal products. On the other hand, unique strains such as isolate BA2968 probably have been introduced to Finland much earlier as evidenced by a high number of single nucleotide variant sites in their genomes.

**JO 04**

**Acinetobacter baumannii as a pathogen of war: New insights from whole genome sequencing**

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**Background:** Over the past 15 years, *Acinetobacter baumannii* has emerged from virtual obscurity to one of the most common causes of nosocomial infections worldwide. One of the seminal events in the emergence of this species was the Iraq war, where at the height of the conflict a new *A. baumannii* infection was reported daily. The severity of these infections was further exacerbated by the multi-drug resistant (MDR) phenotype exhibited by this species, where a combination of intrinsic and acquired resistance to antibiotics severely complicated treatment options. Despite extensive research into this species, there is still a paucity of information regarding the epidemiology of strains involved in this conflict.

**Methods:** Whole genome sequencing (WGS) was performed on 2,467 clinical isolates of *A. baumannii* collected from 2003-2017. The isolates were cultured from all major facilities along the evacuation route from Iraq, including strains isolated from environmental locations in Iraq. Core genome multi-locus sequence typing (cgMLST) and single nucleotide polymorphism (SNP) based analyses were performed to provide greater insight into their epidemiology.

**Results:** WGS revealed a wide diversity of different *A. baumannii* strains and 65 different traditional MLSTs were identified. However, >85% of isolates could be assigned to just 9 different traditional STs, with ST-2, ST-3, and ST-25 being the most common. cgMLST refined these data further and revealed identical strains circulating within and between different facilities. Notably, some isolates
cultured from the environment in Iraq were genetically identical to clinical isolates, suggesting that potential genome engineering. Engineered pathogens were the most common strains identified.

**Conclusions:** Implementation of cgMLST to investigate the epidemiology of A. baumannii from military healthcare facilities revealed a remarkably complex web of transmission networks that spanned multiple geographic areas. Despite the large number of strains involved, the majority of infections could be traced to just 9 clones that circulated throughout the evacuation chain for years. Notably, remnants of these strains are still circulating among recent A. baumannii infections, suggesting that potential reservoirs of these isolates are still present.

**JO 05**
**Risk control in synthetic biology: Predicting pathogenicity of novel DNA sequences with interpretable neural networks**

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Recent developments in synthetic biology lead to emergence of new biosafety threats. Gene editing and genome engineering techniques are dual-use technologies that may be used to redesign and modify pathogens for malicious use. Possibility of modifying bacteria to make them more dangerous to humans was recently recognized in the US as a threat of the highest concern in a consensus study report by the National Academies of Science, Engineering, and Medicine. Risk mitigation policies must be developed by the public health authorities. Prevention is currently dominated by voluntary screening of ordered sequences at DNA synthesis facilities. However, synthetic oligonucleotides shorter than 200 bp are commonly not screened at all, even though they can be easily used to assemble bigger constructs conveying potentially dangerous traits. What is more, traditional approaches to both screening and outbreak analysis are based on those used for detection of natural pathogens or Cold War Era biological weapons. They heavily depend on black lists and databases of known pathogen genomes. This is insufficient in the age of increasingly accessible genome engineering. Engineered pathogens differing from the known ones may evade detection both at the screening stage and after they become a public threat. Thus, risks posed by the unexplored majority of the DNA sequence space need to be assessed. To this end, pathogenic potential can be estimated with machine learning approaches even from individual DNA sequences in the length range of a long oligonucleotide or a short sequencing read. This allows both screening of synthetic sequences before their synthesis and rapid detection of novel pathogens in a metagenomic sample or an isolate. We present a new method based on deep neural networks and show that it enables faster and more accurate predictions compared to the previous approach using random forests. This is especially important in the case of an outbreak or an attack, when a delayed response may cost human lives. We ensure identical predictions for both DNA strands by reverse-complement parameter sharing in convolutional and recurrent neural networks. To provide a degree of interpretability of a learned model, we identify and visualize sequence motifs contributing to the final classification decision, showing that it is possible to train a network to recognize features that would not be used by a human expert. Investigating the relation between particular subsequences and class membership leads to identification of prospective markers of pathogenicity in a human host.

**JO 06**
**Down to the limit – Challenging sequencing technologies with complex samples**

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Profiling the microbial community and detecting highly pathogenic microorganisms in environmental sample are key factors in public health systems, ensuring e.g. the quality of the food processing facilities or allowing the early detection and notification of both, disease outbreaks and presence of terrorist agents, necessary for an effective and rapid control strategy. In the last decade, different sequencing technologies became of high interest for the analysis of environmental samples. However, the detection of highly pathogenic microorganisms, present in low abun-
dance and in highly complex environmental samples (containing a high number of different species which in addition maybe closely-related to pathogens), is still a significant challenge.

To strengthen our diagnostic capability, sewage water, spiked with different but defined amounts of B. thuringiensis, B. anthracis, B. cereus, F. tularensis, Y. pestis and B. melitensis, was used. The extracted DNA was sequenced with deep-sequencing (NexSeq, Illumina) and long-range DNA sequencing (MinION, Oxford Nanopore) technologies in order to combined error-correction to create hybrid assemblies.

The ability of several state-of-the-art bioinformatics tools to identify the spiking material in the sequenced dataset was tested. Sensitivity and specificity of the methods were evaluated in terms of read numbers assigned to genus, species and strain level. For benchmarking, the extracted DNA was additionally investigated using strain or genus-specific quantitative Real-Time PCRs and digital droplet PCRs, which are actually the most sensitive methods for specific DNA quantification.

Results indicate that even for highly complex environmental samples like sewage water, the combination of hybrid error-correction and assembly methods is a suitable and a comparable sensitive method to quantitative PCRs. Furthermore, it allows the identification of a species at the strain level paving the way to the identification of genetic modify organisms. Not the less, the use of hybrid assemblies offers the great possibility to detect virulence and antimicrobial resistance genes in the metagenomic dataset and to help identify the potential microbial source.

JO 07
Development of an automated bioinformatics pipeline and assessment of public sequence databases for identifying non-human biological material using DNA barcoding

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Crime laboratories routinely receive evidence which contains non-human biological material. If identified, such material could help reduce the search area in provenance cases or aid in the identification of a plant toxin. In biosurveillance operations for biodefense, collection and accurate identification of biological materials can be vital. For species-level identification, the unknown DNA sequence is compared to a reference database. The use of short segments of DNA that are accepted by the scientific community for species identification, called DNA barcodes, were used in this work for identifying the source of biological material.

There are two main public sequence databases containing barcode data, the Barcode of Life Data Systems (BOLD) and GenBank, the latter for which the data is not curated. In this study, an initial assessment of both the quality and reliability of the DNA barcode data contained in these databases was performed, a prerequisite to their use in a forensic or biodefense setting. To achieve this, curated reference material for plants, macro-fungi and insects was sourced from national collections, with taxa chosen based on their inclusion in BOLD (total n, 150). The relevant barcode sequences from these reference samples (rbcL, matK, trnH-psbA, ITS and COI) were generated and used for searching against both databases. The ability of each database to obtain the correct taxonomic assignment (genus and species) was assessed, when using the default search parameters: GenBank out performed BOLD for species level identification of insect taxa (43% and 29%, respectively) whereas both databases performed comparably for plant and fungal taxa ( 78% and 57%, respectively). Considering that the correct match was often not discernable among the top matches, modified searches were performed against each database to assess whether resolution improvements were possible.

A bioinformatics pipeline using CLC Genomics Workbench (v.10.0.1) was developed for automated trimming and assembly of DNA barcode sequences. This pipeline outputs a consensus sequence in FASTA format that can be automatically searched against GenBank using a Python script. Comparable length of edited sequence as well as accurate genus and species level identification were obtained between manual and automated pipelines.

This poster will outline the optimal search parameters that should be used when searching an unknown barcode sequence against either BOLD or GenBank database.
Reducing global biological threats in a spirit of partnership: The Federal Foreign Office’s engagement in biosafety and biosecurity

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The deliberate misuse of highly pathogenic agents can be a risk not only for individual countries, but for entire regions. Dangerous pathogens do not respect borders. Their proliferation is fuelled by global trade and growing mobility as well as continuous dual use research of concern in the field of life sciences. Severe conflicts and international terrorism add to the increase of biological threats. Posed challenges call for cross-border cooperation in a spirit of partnership to effectively lessen these security risks on a global scale.

Germany is committed to minimising biological risks. Its sustainable and transnational engagement in the field of biosafety and biosecurity contributes to a safer world. In this context and within Germany’s preventive security policy, the Federal Foreign Office advances partner countries’ capacities through its German Biosecurity Programme and the Federal Government’s Enable and Enhance Initiative.

The German Biosecurity Programme was initiated in 2013. It contributes to the G7 Global Partnership against the Spread of Weapons and Materials of Mass Destruction, and aims at raising awareness and reducing biological risks. Five leading German institutions have been charged with implementing activities in 12 partner countries and two supraregional projects. These activities focus on training measures in the areas of biosafety and biosecurity, surveillance, detection and diagnostics, awareness raising, networking and capacity development.

The efforts within the Federal Government’s Enable and Enhance Initiative were launched in 2016. They aim at strengthening national security capacities and enabling partners to react properly to regional biological threats. Project activities are implemented in seven partner countries. The initiative is coordinated by the Federal Foreign Office and the Federal Ministry of Defence.

Germany’s commitment in the field of biosafety and biosecurity is helping numerous partner countries to develop necessary capacities to protect themselves against potential bioterrorist attacks and other ways of deliberate misuse of dangerous pathogens. In so doing, global non-proliferation of highly pathogenic agents is reinforced.

Preparing for surprise in biodefense

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The U.S. Chemical and Biological Defense Program remains focused on preparing to address new, emerging pathogens as well as potentially genetically modified pathogens on future battlefields. The recent National Academy of Science study, “Biodefense in the Age of Synthetic Biology”, provided several recommendations for the U.S. Department of Defense (DoD) which will be addressed. A brief overview of current U.S. DoD biodefense strategy and research will also be discussed.

What is GOARN?

The Global Outbreak Alert and Response Network (GOARN) is a technical partnership, established by the World Health Organization (WHO) in 2000. In December 2017, the Global Meeting of GOARN partners endorsed the major elements for further development of and operations of the network - an initiative referred to as “GOARN 2.0”:

GOARN is a network of institutions and stakeholders to improve disease event detection, international coordination and response at local, regional and global levels; with access to technical support and
Recent national and international events highlight the importance of preparedness and training of police and health authorities for an effective response to terroristic attacks. During October 10-12, 2017, police and health authorities in Berlin therefore conducted the first multi-agency full-scale bioterrorism exercise called “BAO Wunderbaum” in Germany. Bioterrorist attacks create a complex threat environment with a possible high extent of damage. As such events are also quite rare, there is little experience in the management of such situations among the authorities involved. Since subsequent attacks are likely to occur leading to further possible releases of biological agents, only the fast localization and arrest of the suspects can eliminate the source of infection. Necessary measures from law enforcement and public health often have conflicting priorities, but are of equal importance and must be implemented in parallel. Consequently, the personnel capacity and expertise required to manage such crime scenes, as well as the complexity of possible scenarios, require not only a close cooperation, but rather a joint approach and a spirit of trust between health and security authorities.

The highly complex exercise “BAO Wunderbaum”, involving all relevant management and operational structures of police and health authorities, is unique in Germany and, as far as we know, also in Europe and North America. More than 300 operatives from the security and public health sector and 20 actors participated in the two main exercise days. The exercise itself was based on a realistic scenario in terms of intention and ability of perpetrators to carry out an attack. All measures were performed under a high degree of realism and without previous training: police counter-measures and law enforcement in a contaminated crime scene, identification of the pathogens and prevention of the spread of infectious diseases, dealing with affected residents and with a fugitive offender, medical care of a seriously injured and infectious offender in a special isolation ward, as well as autopsy of an infectious corpse.

The State of Berlin, supported by Federal Governmental Authorities, has shown itself to be a pioneer in the field of combating terrorism in connection with biological hazardous substances in Germany. The experience gained in planning, preparation and realization of this exercise proved indispensable for an effective response to bioterrorism incidents.

**KO 04**
Managing a bioterrorism incident - Lessons learned from the first German multi-agency full-scale exercise in Berlin

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**KO 05**
Education and capacity building: The best approach to bioterrorism prevention and response

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Coordinated multisectoral national and international efforts on raising awareness about a global security, medical, economic, political, sociological challenge as it is today bioterrorism, present the first level in prevention strategy against this threat. The education of the scientific communities and decision makers using modern tools such as e-learning and practical interactive approaches, increased safety of databases, real support to the BTW convention, increased intelligence and early warning, real response strategies and policies are necessary steps. All mentioned includes control of transport and handling of potential B agents and dual-use goods, improvement of biosecurity and biosafety standards, epidemiological surveillance, improvement of procedures of detection and identification of potential B agents using state of art molecular-genetic procedures, enforcing the capacities for medical care of exposed and/or ill persons, as well as the application of all crisis management and crisis communication tools. The military medical sector is of great importance, due to the level of expertise, organizational and resource capabilities to work in emergency situations. The aim of this work is to present Serbian approaches in this field as well as to present the multimedia distance learning e-courses Bioterrorism-Prevention and response that include all previously mentioned.

LO 01
“Little Exercise on the Prairie” – Integration of deployable science with operations

SA Holowachuk
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NATO Exercise Precise Response (Ex PR) is a comprehensive CBRNe exercise that has ran annually in Canada since 2004 and has trained over 4000 CBRNe operators. Ex PR ensures that the operators are able to respond to a myriad of threats in the CBRNe domain, whilst enabling interoperability amongst Nations. Initially Ex PR featured participating Nations working individually through traditional style scenarios and threats, but has evolved into task force led teams to facilitate exchange of techniques, practices, and information. However with the continual progression of the asymmetric threat it has advanced to include intelligence driven scenarios with increasing complexity. This has been marked by advances in technology, both complementary and disruptive, which have required the specialized units to progress to overcome these advances. Although this has led to better kit and overall response capabilities, it has not been without challenges. This is particularly noticeable in the biological domain where field kit is typically unavailable, or ineffective relative to the other disciplines. This requirement for increased knowledge and understanding from operators to be successful in executing a biological mission continues to test units within the Alliance. As a result, there has been a significant increase in the use of “deployable science” to attempt to augment the aptitude of CBRNe teams. This “deployable science” has been integrated through a number of means including: scientific reach-back, deployed scientific advisors or subject matter experts, and through field deployed laboratories. The effective employment of these mechanisms has unequivocally increased the readiness of operators and their ability to respond effectively in theatre. This talk will review the history of Ex PR with a focus on the emergence of "deployable science".

LO 02
How to build a mobile lab for genomic surveillance of viral outbreaks

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Genomic surveillance is a technique that is becoming increasingly important for detecting and responding to viral outbreaks. It requires the sequencing of many viral isolates allowing the use of phylogenetics to provide a high-resolution view of the outbreak. The technique is particularly powerful if it can provide feedback in real-time which is best achieved by deploying a mobile lab equipped with real-time sequencing technology into an ongoing outbreak. The MinION (Oxford Nanopore Technology) is the currently the only such real-time
sequencer portable enough to be used in the field. The sequencing equipment and all required consumables can fit in bags that can be transported in standard airline luggage.

To obtain sufficient material for sequencing we have developed a multiplex PCR method known as Primaseq which given a set a reference genomes, will design a set of primers optimised for amplification of low viral titre samples. Multiple samples can be barcoded and run on a single MinION flowcell reducing the labour and sample cost to under $100 a sample in materials. We have validated SOPs for building a mobile lab, sequencing protocols, nanopore bioinformatics and phylogenetic analysis which can be adapted for other viruses (http://artic.network/resources.html).

We have demonstrated that a mobile laboratory can be flown into an ongoing outbreak and provide rapid, actionable results from the field and avoiding the delays associated with shipping samples. We have deployed mobile labs to Guinea during the 2014 West African Ebola outbreak[1], to Brazil during the 2015 Zika outbreak[2] and are on standby to provide support in any future outbreaks as part of the Wellcome Trust funded ARTIC network project. Further work is required to improve the sequencing methods for faster answers and to allow untargeted sequencing not requiring prior design of validated PCR primers for every virus. Recent progress has been made on fast, efficient basecalling and alignment algorithms for nanopore bioinformatics on portable computer hardware but further work is needed on phylogenetic reconstructions which are typical computationally intensive and visualisation tools for epidemiological interpretation.

References:

**LO 03**

**Pulse controlled amplification: a platform technology for portable molecular detection in 15 minutes**

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For molecular detection to be applied in the field, current integrated systems are typically too complex, bulky, and slow.

We present a battery-operated prototype platform that performs Real-Time PCR with Pulse Controlled Amplification. This technology allows for intrinsic sample purification on a disposable chip and real-time detection within 15 minutes. Data is presented, showing an assay targeting a single-copy gene of Staphylococcus aureus. CFUs were spiked into human full blood as a highly challenging matrix, and quickly lysed. With five minutes of subsequent hands-on-time of on-chip sample preparation, 10 copies of the target per reaction were be detected in 15 minutes total.

The next development steps comprise integrating the lysis of the bacteria and eliminating hands-on steps.

**LO 04**

**Development of low cost, low SWAP sensors for use on mobile platforms**

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The U.S. Department of Defense is developing low cost point sensors to meet reduced size, weight, and power (SWAP) requirements for the detection of biodefense related targets using mobile platforms. Current biological detection methods rely primarily on laboratory based analytical equipment that requires trained users to perform sample preparation, sample analysis, and back-end data analysis. This process is time consuming, requires trained users, and is not well suited for mobile platform based detection. However, for mobile bio-reconnaissance applications, high fidelity answers that come from traditional analytical instrumentation may not be necessary as a simple hazard/non-hazard indicator is sufficient to warn of a plume release or terrain contamination.

The Defense Threat Reduction Agency (DTRA) is currently developing low cost, low SWAP sensors for use on mobile platforms for reconnaissance applications. These sensors will provide rapid detection (e.g., <15 min), provide results remotely via secured wireless communication, and will be platform agnostic. To achieve these goals, sensors will be completely automated from sample collection and preparation, through analysis and reporting. Success will result in sensors for both on-board platform detection applications and one-time-use sensors that can be released (i.e. dispersed over a defined area) from unmanned aerial systems (UAS) to interrogate additional airspace and supplement the UAS platform detector. The fully integrated one-time-use sensors would not require recovery and would be threat agent specific allowing for customizable detection based on region or threats of interest.

The all-inclusive nature of the mobile bio-
reconnaissance sensor will provide mobile platform flexibility to meet end-user needs and will lend itself to be applicable in a multitude of battlefield use cases.

LO 05
Rapid test for the detection of Yersinia pestis F1 capsular antigen in clinical samples for the diagnosis of plague – development, optimization and validation

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Yersinia pestis, the etiologic agent of plague, has caused multiple pandemics. At a temperature of 37°C, Y. pestis expresses the F1 capsular antigen (F1 CA). Thus, the detection of F1 CA with easy-to-use and rapid tests could be a tool in point-of-care settings to confirm or to exclude an infection of patients with Yersinia pestis. An immunochromatographic assay, also known as lateral flow assay (LFA), was developed for the detection of F1 CA in human sera and whole blood samples. First steps of assay development included the screening of several antibodies followed by testing and optimization of raw materials, e.g. nitrocellulose membranes, conjugate pads and sample pads. As the separation of whole blood cell is a crucial step of human diagnostic rapid tests, the challenge of the development comprised the adaption to the test system for whole blood samples. Against this background, our study focused on testing of several filter materials for the separation pad as well as the optimization of both blocking buffer and dilution buffer. In the final test format, the test can be carried out by adding one drop of whole blood or 2 drops of serum to the sample port, followed by 4, respectively 3 drops of dilution buffer. The test result is observed within 20 min, either with the naked eye, or, for quantification, with a portable reader. To date, the detection limit of the LFA was observed to be approx. at 2.5 ng/ml. After in-house validation, cross reactivity was tested at the InstMikroBioBw. LFA showed no cross-reactivity with e.g. Yersinia enterocolitica, and Yersinia pseudotuberculosis.

Epidemiology of tick-borne encephalitis in Germany in 2017

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Tick-borne encephalitis (TBE) is the most important arboviral disease in Central Europe. The epidemiology of the tick-transmitted TBE virus is poorly understood and the numbers of human cases varies more than twice in different years. Detecting new trends in the epidemiology of TBE in Germany will help to focus surveillance and prevention methods in the mainly involved regions. Human cases of TBE were registered with the support of the Robert Koch Institute and the Bavarian Federal Office of Health and Food Safety. Human cases were contacted and followed to identify the location of tick infestation and ticks were collected to isolate and characterize the TBE virus strains. In 2017 a total of 485 human cases of TBE were registered, the second highest number reported since 2001. In Bavaria 234 human cases were reported, the highest number of human cases since the introduction of the German Law against Infectious Diseases in 2001. Bavaria and Baden-Württemberg were the federal states with the highest reported human case numbers. However there are clear spatio-temporal differences on a regional and local level. In the northern parts of both federal states human cases decreased while in the southern part along the Alpian mountains an increase was reported. The responsible factors are still unclear but may include climatic and socilogic factors. TBE is a disease with changing epidemiology in Germany. Following and understanding these changes will help to focus re- sources and prevention efforts to the main involved regions. In some districts TBE has to be named an emerging viral infection as there is a clear pattern of emergence.
MO 02
Clinical aspects of TBE, diagnostic imaging, pediatric disease and potential long term sequelae

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Tick-borne encephalitis (TBE) is the most important tick-borne viral disease worldwide. About 10,000-15,000 cases are reported from Europe and Asia annually. Up to date no effective antiviral treatment of the disease is available. The mainstay of prevention is highly effective TBEV vaccines. However, adherence to vaccination recommendations varies greatly in Europe and in particular in Germany. The review will present current data regarding diagnostic imaging in TBE and radiological risk factors. Further, there is growing evidence, that there are neurocognitive sequelae in children affecting learning tasks, whereas somatic long term sequelae are only infrequently reported. Pediatric outcome studies from European countries and small case series will be demonstrated. Issues of pediatric vaccination strategies will be discussed.

MO 03
Encephalomyelitis caused by tick-borne encephalitis virus (TBEV) in the second trimester of pregnancy: Severe affection of the mother and normal outcome of the child

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Tick-borne encephalitis virus (TBEV) is endemic in large parts of the European continent. Despite the high incidence of annual symptomatic TBEV infections, little data is available about the course of TBEV infections during pregnancy and their implication for fetal health, birth and the development of the child. We report the case of a patient at 19 weeks gestation who presented with acute fever, headache, signs of meningism and tetraparesis. Acute TBEV encephalomyelitis was diagnosed by cerebrospinal fluid (CSF) analysis showing elevated leukocyte count, and by positive TBEV serology without prior TBEV vaccination. Due to progressive cervico-pontine myelitis, tetraplegia and subsequent respiratory insufficiency, the patient was referred to intensive care unit and underwent invasive ventilation 5 days after admission. Over the course of 2 weeks the patient’s condition stabilized and weaning was initiated. Under daily physiotherapy, the tetraparesis was slowly regressive and the patient was ultimately able to walk with little support. Fetal biometric measurements and Doppler wave-form analysis of the umbilical artery were started at 24+3 weeks gestation and showed normal values throughout gestation. No relevant gestational complications were observed. The neonate was born by cesarean section at 38+4 weeks gestation. During 7 month postnatal follow-up, the development of the child revealed no signs of neurological impairment. Umbilical cord blood of the newborn was negative for TBEV-DNA. TBEV-serology of the child showed antibodies of the IgG-isotype which decreased over time and that thus is compatible with materno-fetal transferred IgG-antibodies. Despite the severe clinical affection of the mother in this case, the TBEV-infection was apparently not transmitted to the fetus and did not impair its health and development. Naturally, further studies and case reports are needed to adequately assess the risk of acute symptomatic or asymptomatic TBEV-infection during pregnancy.

MO 04
News about ixodid tick species (Acari: Ixodidae) in Germany

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Of the 54 ixodid tick species known in the western Palearctic, 18 tick species from the genera Ixodes, Dermacentor, Haemaphysalis and Rhipicephalus have been reported in Germany. Of these, the common Ixodes ricinus in many regions of Germany accounts for more than 95% of the tick fauna. However, intensive morphological studies showed that in addition to I. ricinus, several other Ixodes species are more widely used than previously assumed (e.g. Ixodes frontalis, Ixodes inopinatus). Furthermore, these studies show that Ixodes species exist in Germany that have not been described so far, as Ixodes (Pholeoixodes) cornatus was discovered in Baden-Württemberg on martens (Mustela putorius), a tick species that was previously described exclusively in Tajikistan. In addition, in recent years
Rhipicephalus sanguineus ticks have been repeatedly discovered in flats in Baden-Württemberg. This tick species was presumably introduced by dogs from the Mediterranean region and could develop into housing populations. A Hyalomma rufipes male and several Hyalomma marginatum males and females were also proven to be at least temporary part of the German tick fauna which were probably imported from the Mediterranean region or from Africa via birds. A clear identification of these types of ticks is particularly important as they have a very different medical and veterinary significance.

NO 01
Pasteur Institute of Tunis and Robert Koch Institute of Berlin: a successful collaboration challenging biosecurity issues

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The Collaboration between the Pasteur Institute of Tunis (IPT) and the Robert Koch Institute (RKI) started in 2014 as part of the ‘German Biosecurity Programme’ engagement within the Global Partnership of the G7 countries, where Tunisia is a focus country for the 2 phases: 2014-2016 and 2017-2019. Emerging pathogens and recent outbreaks have shown that highly contagious diseases can threaten the health and the safety of populations as well as the stability of states and societies. Such diseases can spread naturally or through a misuse of pathogenic biological materials. In this context, the common objectives are to promote international standards of biosafety and biosecurity and to strengthen detection and diagnostic of highly pathogenic agents at IPT, as the national reference institution for health research and diagnostics.

First, the joined organization, by the Tunisian and the German experts, of training courses in biosafety and biosecurity for all IPT staff and doctoral school of Tunis, has enabled the setup of a permanent training program at IPT. The RKI has also supported financially, the conception of adapted teaching tools for maintenance and cleaning personnel of the IPT.

This experience is unique in Tunisia as it was carried out by involving IPT workers, experts and hygiene managers and might represent a transferable model to other Tunisian health institutions.

Then, the improvement of the institution capacities for biosafety in the different IPT laboratories for the diagnosis of highly pathogenic viruses and bacteria has been the output of IPT/RKI collaboration by installing a glovebox, organizing training for its use correctly, and setting up new molecular diagnostic assays.

Moreover, as part of its involvement for better address of biohazards, IPT is conscious of the need to provide a code of conduct (CoC) allowing researchers to assess any potential risk associated with their research work. This CoC was a result of a strong collaboration between biorisk experts from IPT and RKI, as well as IPT’s Biomedical Ethics Committee members. This CoC applies to research in all the areas of IPT activities and defines the procedure for assessing a potential dual use in order to preserve beneficial research to the society and, above all, prevent dissemination of harmful information and results.

The challenges have just begun and therefore we will increasingly be called to unite strengths, knowledge, expertise and resources.

NO 02
Management of biological risks in Sudan: A promising integrated approach

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Within the framework of the German Biosafety & Biosecurity (B&B) measures with Sudanese institutions on national, state and institutional level. This includes a concerted approach of training and implementation of a management system to mitigate the risks inherent to handling of biological agents.

Key implementation partner is the Sudanese National Public Health Laboratory (NPHL). It is the executing institution of the Federal Ministry of Health (FMoH) and coordinating body of all State Public Health Laboratories (SPHL). NPHL plays a significant role in managing disease outbreaks in Sudan.

In a first program phase, participants from eight different Sudanese institutions incl. NPHL, from the area of the capital Khartoum, were trained as B&B trainers. This pool of trainers functions as multiplier and implementer for B&B in current activities. In the ongoing second phase, biorisk officers from state public health or major hospital laboratories of State MoHS are trained as B&B multiplier during a training-of-trainers (ToT) workshop series, including didactics training. The B&B content is adapted to Sudanese context and facilitated by phase 1 participants, with a joint Sudanese-German coordination and technical support. This comprehensive theoretical and practical B&B training and implementation in between workshops builds the foundation for B&B education in Sudanese state institutions.

Based on the acquired knowledge of phase 1, the setup of a biorisk management (BRM) system at NPHL (according to CWA 15793) is supported. It sets a managerial framework and enables the institute to effectively identify, monitor and control laboratory B&B aspects. This activity started with a GAP-analysis, setup of action plans, implementation of priority topics and a regular conduction of audits. The principles of the BRM System are envisioned to be transferred to state public health labs in the future.

This approach is complemented by awareness raising with decision makers on national and federal level. Together, the successful activities support the transfer of B&B knowledge on all levels and the setup of a framework for implementation of a biorisk management system. It furthermore supports the establishment of a strong network between state biorisk officers with NPHL, biorisk management department, thus strengthening a national capacity and sustainable implementation of a B&B culture in Sudanese institutions.

**NO 03**

**Strengthen global networks to identify the biological agents - Building a network of laboratories in Pakistan, Egypt and Ukraine to enhance biosafety and biosecurity**

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The unprecedented pace of global scientific development, the dual-use nature of biological materials and technologies and the ambition of terrorist groups to launch biological attacks, combine to contribute to the significant international security threats posed by biological terrorism. Biological agents pose unique threats to global security given their naturally occurring and self-replicating character. Brucellosis, Glanders and Q-Fever are contagious zoonotic disease of animals with significant economic impact and public health concern.

The current project is international cooperation between Germany represented by Friedrich-Loeffler-Institut and Pakistan, Egypt and Ukraine represented by scientific research institutes and academic universities. The project funded by The Federal Foreign Office in Germany from 2017 to 2019. The project focuses on zoonotic bacterial infections in livestock and humans in these countries. The effort will be directed to the accurate diagnosis and will be directed to develop effective strategies to combat and control these diseases in both animals and humans.

Pillars of our approach are training of veterinarians and human health professionals from Pakistan, Egypt and Ukraine in the implementation of prevalence studies, typing of isolates to detect chains of infection and improvement of biosecurity and biosafety measures in order to raise awareness.

The samples will collected according to statistical plan from different animal species and humans in different districts. The samples were firstly investigated serologically and by molecular tools in partner countries laboratories and then transported to FLI in Germany for further investigations.

This bundle of the project activities will have positive effects through raising biosafety awareness of biological agents, improving the income of small farmers in undeveloped rural areas and will improve the biosecurity situation in the country.

Further profit of the project to draw an epidemiological plane in this country and make an optimum design to combat and control brucellosis in order to improve public health.
NO 04
G5 Sahel biosafety network: Challenges and prospects

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Introduction: Terrorism is rampant in Africa, particularly in the Sahel region, where attacks are frequent. This Sahelian band of Africa constitutes an eco-climatic peculiarity that favors the emergence of epidemics of infectious diseases. The cerebrospinal meningitis belt is located in this region. In 2016, an epidemic of rift valley fever was reported in northern Niger and a few cases were reported in northern Mali. Dengue epidemics have been reported in Burkina Faso in 2016 and 2017. This region is poor and desert with displacements of populations and cattle which offers favorable conditions to the terrorist organizations. The risk of bioterrorism is real even though many people think it is weak. To fight terrorism effectively, Burkina Faso, Mali, Mauritania, Niger and Chad have decided to create the G5 Sahel. This structure aims to allow the resilience of populations. In November 2017 G5 Sahel public health institutions (Burkina Faso, Mali, Mauritania, Niger and Chad) in partnership with Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, Institute of Microbiology of the German Armed Forces (IMB), Fondation Mérieux, have decided to create a G5 Sahel biosafety network. The network was created and is coordinated by the Infectiology Center Charles Merieux-Mali.

Results: Two biologists per country have been trained. A study tour to Germany allowed members of the Network to view the response plan in the event of an outbreak by a highly dangerous pathogen. The G5 mobile laboratory was set up at CICM-Mali and equipment was arranged in each of the other 4 G5 Sahel countries. Two series of trainings on the detection of dangerous pathogens have been organized.

Challenges and Perspectives: Ownership by the G5 Sahel authorities is an important issue. Maintaining skills at the level of each institution is critical to eventually forming a G5 Sahel team for rapid functional intervention. Procedures for deploying the mobile lab remain to be developed. For the moment Germany supports the network in its operation but its long-term maintenance is a major challenge.

NO 05
German Online Platform for Biosecurity & Biosafety (GO4BSB): Raising biological risk-related awareness through e-learning programmes

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The German Online Platform for Biosecurity and Biosafety (GO4BSB) is the e-learning platform of the German Biosecurity Programme, which was launched in 2013 by the Federal Foreign Office under the auspices of the G7 Global Partnership against the Spread of Weapons and Materials of Mass Destruction. It is a collaborative initiative between the Robert Koch Institute (RKI), the Bundeswehr Institute of Microbiology (IMB), and the Friedrich-Loeffler-Institut (FLI), and managed by the Bernhard Nocht Institute for Tropical Medicine (BNITM).

GO4BSB has been established to raise awareness about biological risks, including the potential misuse of pathogens, toxins, as well as bioscience-sensitive information and technology. To this end, e-learning tools and materials provided on GO4BSB enable learning about responsible conduct in the life sci-
ences and management of biological risks. Additionally, the platform encourages inter-disciplinary scientific exchange, which goes beyond the limitations of physical classroom settings. GO4BSB is based on both the concept and the design of virtual learning environments commonly used in higher education. In order to address challenges related to the potential misuse of sensitive information, GO4BSB requires registration and consists of different access levels ranging from shared to restricted areas (‘Course rooms’). Conceptually, GO4BSB in collaboration with individual project partners offers tailored, context-specific e-learning programmes in four different languages (English, French, Russian and German) to overcome language barriers. GO4BSB-based programmes are currently being implemented in Tunisia, Kosovo and the Ukraine. Further programmes for Cameroon, Mauritania, Kenya, Rwanda, Burundi, South Sudan, Tanzania and Uganda are currently being set up.

In conclusion, e-learning can be an important tool for raising awareness about biological risks and their management, including the necessity of responsible conduct in the life sciences. Context-specific, multilingual teaching and learning materials provided online are cost-effective and have a wide geographical reach.

NO 06
Building biosecurity capacities through advanced fellowship programmes: The example of the Global Partnership Initiated Biosecurity Academy for Controlling Health Threats (GIBACHT)

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The Global Partnership Initiated Biosecurity Academy for Controlling Health Threats (GIBACHT) is a multilateral one-year biosafety and biosecurity training programme. GIBACHT started in 2014 and is currently running its fourth cohort. It targets postgraduate public health professionals of partner countries in Africa, the Middle East, and South and Central Asia. The programme is supported by the German Biosecurity Programme and conducted in partnership between the Bernhard Nocht Institute for Tropical Medicine (BNITM), the Robert Koch Institute (RKI), the Swiss Tropical and Public Health Institute, and the African Field Epidemiology Network (AFENET).

GIBACHT aims to provide knowledge and skills on intentional and unintentional infectious disease threats and their control. In order to strengthen national capacity in disaster management and preparedness, GIBACHT sensitises for biosafety and biosecurity issues including „dual-use“, and promotes the establishment of sustainable international co-operations in disease prevention. The training consists of 20 e-learning modules, three face-to-face workshops (in Hamburg, Berlin, and Kampala/Uganda), and distance-based group work to develop teaching materials (case studies), with a total workload of around 180 hours for the fellows. GIBACHT is responding to the need of countries with insufficiently prepared health systems and risk of accidental and deliberate release of infectious agents. To date, 64 fellows from 19 countries have been trained. In the second funding phase, the programme was adapted to put greater emphasis on sustainability of the newly built capacities. In line with the multiplier concept, fellows are conducting case study exercises in their home institutions. Beyond this, many have established biosafety/biosecurity trainings in their home countries. The knowledge exchange is further strengthened by the implementation of a Moodle-based alumni network and inclusion of GIBACHT alumni as workshop facilitators and at international conferences. Anonymous evaluations of the course content by the fellows from the first three cohorts have shown great acceptance of the programme with scores above 3 (mean of 3.7, n= 43) on a 1-4 rating scale.

In the future, the case studies developed by the fellows during the programme will be shared with the scientific community through publication and by implementation at their home institutions.
**OO 01**

Neurological disease after short travel to Gambia with fatal outcome

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**OO 03**

Unpleasant souvenir from Italy

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**OO 02**

A fatal encephalitis in a young healthy woman

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**OO 04**

A case of a horse disease resulting in death

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**OO 05**

A headache with surprising outcome

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**PO 01**

At the tick-virus-host interface: Elucidating the transmission and pathogenesis of Crimean-Congo hemorrhagic fever virus

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Ticks transmit at least 25% of the known 560 arboviruses, many of which are medically important and have an emerging and re-emerging propensity. Crimean-Congo hemorrhagic fever virus (CCHFV) is a notable example, which causes up to 30% mortality in humans, and is classified as a risk group 4 agent. CCHF has been recorded in more than 32 countries, making it one of the most widespread hemorrhagic fevers of humans; yet, there is no commercially available vaccine and disease is difficult to treat. CCHFV elimination strategies, such as therapeutics, acaricides, and vaccines, have been unsuccessful so far, thereby warranting the develop-
opment of a novel approach. The research in our laboratory aims to understand the determinants of virulence and virus-host-tick interactions. Here, we will give an overview of gaps in knowledge and current progress in understanding transmission and pathogenesis of tick-borne hemorrhagic fever viruses.

**PO 02**

Emergence of West Nile virus in Central-Europe

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**PO 03**

Identification of asymptomatic plague cases and immune response study, Madagascar

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Plague is a zoonosis which affects mainly rodents and caused by the bacterium *Yersinia pestis*. It is transmitted from rodents to humans by the bites of infected fleas. The disease has 2 major forms: bubonic and pneumonic plague. However, the existence of asymptomatic plague cases has been reported. The aim of our study is to identify asymptomatic plague cases and compare their immune response to confirmed plague patients. The study was carried out in 3 plague endemic districts of the central highlands of Madagascar. Populations were randomly selected for individual interview on their exposition to plague risks and for blood sampling before and after one plague season. An asymptomatic plague case was defined as an individual who seroconverted before and after a plague season without antibiotherapy and without contact with a plague patient. Confirmed plague patients (*Y. pestis* strain isolated) and healthy controls were also blood sampled. The study of their humoral immune response was performed using ELISA for anti-F1 IgG detection (DO>1 was considered as high responder whereas DO<1 was low responder). Among 534 individuals enrolled before (2013) and after a plague season (2014), 13 seroconverted but 2 received antibiotherapy. Only 11 were then considered as asymptomatic plague cases. Thirty-three confirmed plague patients from 2000 to 2017 were sampled while 10 healthy controls were also tested. The study of their humoral immune response to plague showed that the persistence of anti-F1 IgG among confirmed plague cases was highly variable, ranging from 1 month to 14 years post-infection. Proportion of high responders (12/22) and low responders (10/22) were similar while identified asymptomatic cases (10/11) were mostly low responders. As expected all healthy controls were negative to anti-F1 IgG antibodies. Although plague is a fatal disease, asymptomatic plague cases also exist in Madagascar. Our results suggest that 2% of exposed population can acquire the disease without showing symptoms. Immune response to plague is contrasting between identified asymptomatic plague cases and confirmed plague patients. We assume that this difference may be related to the severity of the disease. Previous field investigations in Madagascar already mentioned some plague asymptomatic cases but based upon a single positive anti-F1 serology. Our study is the first using paired sera but their role in the epidemiology of plague deserves more investigation.

**PO 04**

Q fever in the USA

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Infection with the bacterial pathogen *Coxiella burnetii* causes the human disease Q fever. *C. burnetii* is considered a potential bioweapon and is classified as a select agent in the United States due to its low infectious dose, aerosol transmission, and stability. Q fever is also an emerging public health threat, with global distribution and frequent outbreaks. In the United States, Q fever has been a notifiable disease since 2000. Reports of Q fever cases to the CDC increased from 2000-2006, but then plateaued with between 113 and 135 cases reported per year for 2008-2012. Notifications of Q fever cases have recently been higher, with 192 cases reported in 2017. Although the reported cases of Q fever are low in the U.S., the environmental burden of the bacteria is quite high, and seroprevalence in the general population is greater than 3%. These data suggest that even though reported cases are low, exposure to the bacteria is common. To better understand the dichotomy between *C. burnetii* exposure and reported cases, we have identified and characterized strains present in the environment, reservoir animals, and human infections. We have
also analyzed serologic testing data from four major commercial laboratories in the United States. The data show that only three C. burnetii genotypes are found in the U.S. environment. One has a close association with cattle, one with goats, and the other does not have a specific reservoir association. Strains associated with human infections are primarily goat-associated, and appear attenuated in vitro and in vivo models compared to the Nine Mile reference strain. Serologic testing data show that greater than 12,000 specimens are tested by these labs each year in the U.S. The majority of these specimens (84%) are negative for C. burnetii antibodies, but the number of positive test results suggests that Q fever is under reported. The data suggest that a combination of under reporting and lower virulence isolates in circulation may lead to a relatively small number of reported Q fever cases compared to the high environmental burden in the U.S.

PO 05
Tick-borne relapsing fever in Nigeria: Candidatus Borrelia kalaharica and its Ornithodoros savignyi tick vector

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Indigenous cases of tick-borne relapsing fever have not been reported from Nigeria despite hospital admissions of patients presenting with compatible clinical signs. In order to further investigate, we studied 49 pooled samples of soft ticks collected from Nigeria. These were predominantly adults and nymphs and were pooled by life stage and DNA extracted in Nigeria using a DNeasy standard protocol. Samples were then shipped to University of East London for further analysis. To confirm the identity of the ticks, multiple primer sets yielding a product, yet all other assays successfully amplified the positive control used (Ixodes ricinus DNA). Sanger sequencing revealed the tick to be Ornithodoros savignyi, an aggressive multi-host feeding species of notable veterinary importance. These ticks were screened for various pathogens including Rickettsia, African Swine Fever virus, Coxiella, Bartonella and Borrelia. Only the latter yielded results with three samples positive using a 16S rRNA genus specific PCR for Borrelia. Attempts to further characterise the species using primers against 16S rRNA, flagellin and intragenic spacer (IGS) regions were assessed by Sanger sequencing, revealing the identity of this species to be Candidatus Borrelia kalaharica. This recently proposed species has been reported to cause relapsing fever in a returning traveller from South Africa to Germany. Notably, this spirochete shows remarkable similarity to an unnamed species reported from Ornithodoros moubata ticks and humans from Tanzania. This could challenge the dogma of “one tick species and one Borrelia” that has been considered the ecological norm for relapsing fever borreliae.

PO 06
Orthohantavirus infections as a cause of fever of unknown origin in Kazakhstan

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Fever of unknown origin (FUO) can be caused by a broad variability of zoonotic infectious agents. Studying zoonotic diseases in Kazakhstan is of great interest as the vastness of the territory harbors many natural foci for zoonotic infections. Aim of this study was to explore the seroprevalence of orthohantaviruses in patients’ sera suffering on fever of unknown origin (FUO) in Kazakhstan. The study was set up in 2014-2015 among patients with FUO in the two selected regions (Almaty, Kyzylorda) in 13 hospitals. Blood sampling was performed twice (day 1, day 7-14). For antibody investigation and serotype determination, commercial ELISA and Immunoblot kits were used. In total 802 sera samples were investigated from Almaty (n=378) and Kyzylorda (n=424) regions. Out of 802 sera samples, 22.2% (178/802) were positive for IgG antibody. Seroprevalence orthohantavirus IgG antibody in Almaty region was 21.2% (80/378) and 23.1% (98/424) in Kyzylorda region. Four patients 0.5% were positive for IgM antibody. Positive samples were tested by Immunoblot IgG and IgM to determine serotypes. Results showed 2.7% (22/802) positive for Puumala orthohantavirus, 1.1% (9/802) Dobrava-Belgrad orthohantavirus and 0.4% (3/802)
Hantaan Orthohantavirus respectively. Immunoblot IgM results confirmed three acute infections with Puumala orthohantavirus and no specific serotype in one case. For the first time it’s shown that orthohantavirus infections might be present in Kazakhstan. The data obtained also indicate that the diagnostics of orthohantaviruses among people with FUO is important. So far data on orthohantaviruses in rodents in Kazakhstan are sparse. Rodent studies are planned in order to characterize the circulating virus strains in the pilot regions.

PO 07
Tick-borne encephalitis virus emerging in mountainous areas

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Tick-borne encephalitis is one of the most important human encephalitic diseases in Eurasia. The etiological agent of TBE is a Flavivirus, and three subtypes of TBE virus the European (western) subtype (TBEV-EU), the Sibirian subtype (TBEV-Sib) and the Far-Eastern subtype (TBEV-FE) are recognized. Since 2002 tick-borne encephalitis has started to emerge in mountainous areas where no infections had been reported before. So far it is unclear whether these changes in epidemiology are due to climatic changes or to viral or social factors. In this study, we used old and recent virus isolates from the Northern and Southern regions of the Alpian Mountains. TBE virus strains from mountainous regions were compared with TBE virus strains from lower altitudes (non-mountain regions) in order to determine differences which might select for better circulation in the mountainous conditions. To address this question growth curve assays were established and the individual growth curves of the alpine and non-alpine virus strains were compared to each other. Moreover, we generated the E genes of the selected TBE virus strains and compared them to each other. So far, our data show that all alpine virus strains belong to individual genetic clades, which indicates different origins of all alpine TBE virus strains. Based on our findings we reconstruct the emergence of new TBE virus natural foci.

QO 01
Crimean-Congo hemorrhagic fever in Kosovo, 2013 - 2018

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Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne disease that has been diagnosed in more than 30 countries in Europe, Asia and Africa. The CCHF infection is caused by an RNA virus of the genus Orthohareovirus, family of the Nairoviridae. Ticks are both reservoir and vector of the CCHF virus. Humans are infected through tick bites or through contact with blood or tissue of infected animals or humans. The Balkan state Kosovo is an endemic area of CCHF in Europe. In the last five years (2013-2018) 33 CCHF cases were diagnosed in humans, 28 (84.8%) males and 5 females (15.2%), with a total of 11 (33.3%) fatalities. These cases were confirmed by ELISA, Immunofluorescence and RT-PCR testing. The vast majority was infected by tick bites, but there were also cases of human to human transmission. Almost all cases were from the central part of Kosovo, the principal location being the municipality of Malisheva (66.7%), followed by neighboring municipalities, Kllina (12.1%), Gjakova (9.1%), Rahovec (6.1%) and Drenas (3.0%). A CCHF case from the year 2018 was reported for the first time from the eastern part of Kosovo, at the Gjilan municipality. The CCHF infections followed a seasonal pattern, most of the cases occurring during the months of June and July. A high viral load (>10^8.5 copies/ml) with the CCHF virus in addition...
to hemorrhagic syndrome in connection with central nervous system impairment were associated with increased risk of death. Treatment with Ribavirin did not have an impact on fatality or survival of CCHF cases.

**QO 02**

**Study of the Rift Valley fever in Northern Cameroon: Entomological investigation, detection and molecular characterization of viral strains**

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Rift Valley Fever (RVF) is an arbovirosis of medical and veterinary importance transmitted by several species of mosquitoes. The expansion of this disease in recent decades, as evidenced by the many epizootics and epidemics observed in Africa, may be due to environmental and climatic changes and vectors abundance. Therefore, an entomological study of vectors population in various ecological sites in the North Cameroon region was conducted from January to November 2016 with the aim of: (i) identifying the main vectors of RVF in the North Cameroon, (ii) detecting and characterizing RVF virus strains within vectors population using molecular techniques. Overall, 2600 mosquitoes were collected during dry and rainy seasons in the localities of Garoua, Pitoa and Lagdo using sheep or goat as bait traps, and bright traps type CDC. These mosquitoes have been identified using dichotomous keys. Four 4 genders (Culex, Aedes, Mansonia, Anopheles) and 20 species with 4 majors (Mn. Africana, Mn. Uniformis, Cx. Poicilipes, Cx antennatus) which have been reported as potential vectors of RVF virus were determined. The dynamics of vectors in their environment varied from one month to another, with a high density from August to October. Detection of the viral genome in these specimens is underway, and of the 600 mosquitoes already tested in PCR, the genetic material of the RVF virus has not been detected. These results will make it possible, to draw up an epidemiological assessment of the disease and possibly to guide the prevention and control actions in the most at risk areas.

**QO 03**

**Seroprevalence of *Francisella tularensis* among wild boars in Ukraine**

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**Background:** Tularemia is an acute, zoonotic infection caused by the aerobic, gram-negative bacillus *F. tularensis*. Tularemia is endemic in most of the European countries, including Ukraine. One of the main reservoirs of *Francisella tularensis* in nature is the wild boar. Hubálek et al. presented 10.8% of tularemia prevalence in sera of wild boars in Czech Republic during 1993-2001 y. The highest (17%) prevalence of tularemia antibodies was found in wild boars during 1993–1994 at the beginning of a widespread outbreak of tularemia in South Moravia that started in 1994.

Serological diagnosis of tularemia is based on the identification of antibodies induced by the immunodominant lipopolysaccharide (LPS) of the *Francisella* cell envelope. ELISA is an easy and fast diagnostic tool, but it requires confirmation by Western Blotting. Most observed cross-reactions causing false positive reactions are attributed to microorganisms such as mycobacteria, *Listeria, Legionella, Brucella, Coxiella* and *Rickettsia*. For decreasing the number of false-positive results and high background in general a competitive ELISA (cELISA) with monoclonal secondary antibodies can be used. Such cELISA diagnostics can be applied to animal and human sera as well. In our case, the wild boar serum was used for screening the probable tularemia natural foci in Ukraine.

**Materials and methods:**

**Serum samples:** A total of 707 sera from wild boars collected during 2011-13 in 20 regions of Ukraine were tested in cELISA. All positive samples were confirmed by Western Blot.

**cELISA:** Competitive ELISA was provided using 1 µg/ml *F. tularensis* LPS (Micromun GmbH, Germany) and secondary antibodies FF27POD (obtained from hybridoma culture in Institute of Microbiology Bundeswehr, Germany). Optical density (OD) was measured using Multiskan™ FC Microplate Photometer, (Thermoscientific). For the data proceeding, the threshold formula was applied as followed: mean – (standard deviation *3) that based on negative control triplet results. All
samples that had values higher than the threshold were considered as negatives.

**Western blot:** Western Blot was performed as the standard method using *F. tularensis* LPS (Mircromun GmbH, Germany) in a concentration of 2.5 pg/μl. LPS was run in polyacrylamide gel with subsequent transfer on nitrocellulose membrane. Rabbit-anti-Pig HRP-coupled IgG (Invitrogen, USA) were used as secondary antibodies.

**Results:** Of the 707 serum samples from wild boars, 303 samples showed the positive result in cELISA, and 87 (12.31%) of them were confirmed by Western Blotting. The major part of collected samples originated from woody parts of Ukraine, as it is the most typical habitat area for wild boars. The highest percentage of positive samples was shown in Cherivtsi and Chmelnytsky regions (51.3% and 44.1%). These regions are located in woody areas with plenty of rivers and periodical floods that may function as a source of water-born tularemia outbreaks. The 216 samples, that could not be confirmed by Western Blot are supposed to have cross-reactivity with relative bacteria.; **Conclusions:** The results obtained in the study demonstrate that tularemia is highly prevalent among wild boars in the Ukraine and therefore the consumption of animal products could be a potential source of infections in humans.

**QO 04**

Proteomic profiling of virulent phase I and avirulent phase II of *Coxiella burnetii* employing axenic and cell culture-based cultivation

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*Coxiella burnetii*, a category B biological warfare agent, causes multiple outbreaks of the zoonotic disease Q fever worldwide, each year, with an increasing tendency. To date, lipopolysaccharide (LPS) is, besides the type IV secretion system the only defined and characterized *Coxiella* virulence determinant.

In this study, avirulent *Coxiella burnetii* RSA 439 phase II and virulent *Coxiella burnetii* RSA 493 Phase I were cultivated both in acidified citrate cysteine medium (ACCM-2) and ACCM-D (defined). In addition, L929 mouse fibroblasts were infected by both *C. burnetii* strains. The cultures were inactivated/lysed, and analysed using liquid chromatography on-line coupled with mass spectrometer employing label free quantification to compare all particular groups.

In axenic media and in L929 infected cells, over 700 and 900 *Coxiella* proteins respectively, were identified. The ACCM-2 and ACCM-D media yielded comparably different proteomes for particular strains. The changes between virulent Phase I and avirulent Phase II did not correspond unequivocally with findings in L929 infection datasets. This study for the first time compared the axenic media and in vivo cultures at the proteomic level. Among tens of candidates for the new virulence determinants, the most prominent roles represent proteins connected with lipopolysaccharide biosynthesis outside the known deleted region from the avirulent Phase II and the proteins connected with the Type IV secretion system (T4SS). From the T4SS functional complex, several Icm proteins were upregulated and together with the finding of up-regulation of PmrAB complex (responsible for the regulation of the most of the T4SS proteins) and upregulation of other secreted effectors in the proteomes of virulent Phase I strain cultured in tissue culture cells, the role of T4SS was emphasized for the first time at the proteomic level.

**QO 05**

Low energy electron irradiation efficiently inactivates pathogens while preserving antigenic structures - A promising novel method for laboratory safety and for the generation of vaccines

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Inactivated vaccines are usually produced by chemical treatment of pathogens, but variations in inactivation efficiencies and extensive downstream processing are major disadvantages of such methods. An alternative inactivation technique is ionizing radiation, which preferably damages nucleic acids, leaving antigenic protein structures largely conserved. In contrast to state-of-the-art irradiation technologies such as gamma-rays, low-energy electron irradiation (LEEI) requires only minor shielding constructions, which enables the use in standard laboratories, including GMP- or high BSL-environments. We evaluated LEEI as an inactivation method for
pathogens in liquid solutions. Different viruses and bacteria were tested for infectivity, genome integrity and antigen conservation before and after LEEI treatment. A dose-dependent fragmentation of nucleic acids was observed, whereas antigenic structures remained largely intact. Animal experiments revealed that LEEI-inactivated viruses and bacteria are capable to induce protective immune responses. Results thus indicate the high potential of LEEI for the production of inactivated vaccines. In addition, LEEI-inactivated pathogens can still be subjected to antigen detection or PCR, which opens the possibility to use LEEI for diagnostics of highly infective material in standard laboratories.

**QO 06**

Phage therapy centre, Queen Astrid Military Hospital, Brussels: Practical clinical aspects

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We have to face the challenge of increasingly resistant bacteria. There are for the moment no new antibiotic drug lines in the pipeline. Other new therapeutic strategies are currently being studied in an attempt to bring the ever increasing problem of antibiotic resistance to a halt, one of these is the use of bacteriophages. The Queen Astrid Military Hospital has a state-of-the-art production capacity for phagetherapy. They adapted the more than 100 years old techniques to a modern production line. In addition, from September 2018, their phage productions will be in agreement with the new Belgian guidelines, approved by the Belgian Regulator (FAMHP). We will present the new practical clinical aspects for treating patients, suffering from multi-resistant bacteria, with phagetherapy in our health-care facility in Brussels.

**QO 07**

Treating anthrax-induced meningitis in rabbits

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Treatment of Anthrax is challenging, especially during the advanced stages of the disease. Recently the CDC updated its recommendations for post-exposure prophylaxis and treatment of exposed populations (pre and post symptoms onset). These recommendations distinguished, for the first time, between the systemic disease with and without meningitis, a common and serious complication of anthrax. The CDC considers all systemic patients as meningeal unless positively proven otherwise. The treatment of patients suffering from systemic Anthrax with suspected or confirmed meningitis includes the combination of three antibiotics – a fluoroquinolone (Levofoxacin or Ciprofloxacin), a β-lactam (Meropenem or Imipenem) and a protein-synthesis inhibitor (Linezolid or Clindamycin). In addition, treatment with an antitoxin (αPA antibodies) and Dexamethasone should also be applied. Since the efficacy of most of these treatments was not demonstrated, especially in meningeal animal models, we developed an Anthrax-meningitis model in rabbits and tested several of these recommendations. We demonstrate that in this model, Ciprofloxacin, Linezolid and Meropenem are ineffective as single treatments while Clindamycin is highly effective. Furthermore, combined treatments of Ciprofloxacin and Linezolid, or Ciprofloxacin and Dexamethasone failed in treating meningeal rabbits. We demonstrate that Dexamethasone actually hinders the blood brain barrier penetration of antibiotics, reducing the effectiveness of antibiotic treatment of Anthrax-meningitis in the rabbit model.

**QO 08**

Molecular and genomic characterization of an equine molluscum contagiosum-like virus

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Cases of pox-like lesions in horses and donkeys have been associated with poxviruses belonging to different genera of the family Poxviridae, including the orthopoxviruses vaccinia virus (VACV) and horsepoxvirus (HPXV), parapoxvirus (PPV) and molluscipoxvirus (MOCV). However, with the exception of VACV and HPXV, genomic characterization of the causative agents remains largely elusive with only single short genome fragments available. Here we present the first near full-length genome sequence of an equine molluscum contagiosum-like virus (eqMCLV) directly determined from skin biopsies of a horse with generalized papular dermatitis. Histopathological analysis of the lesions revealed an epidermal hyperplasia, consisting of an enlarged stratum spinosum with numerous

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large, eosinophilic, intracytoplasmic inclusion bodies within keratinocytes. Virions were detected in the lesions in embedded tissue by transmission electron microscopy. The genome sequence determined by next and third-generation sequencing comprises 161,270 nucleotides (nt) with inverted terminal repeats of at least 670 nt. 21 of the predicted 158 ORFS have no homologues in other poxviruses. Intriguingly, two of these ORFs were identified to encode homologues of mammalian proteins involved in immune signaling pathways, namely SECTM1 and IGFLR1, offering new insights into the vast portfolio of poxvirus immune evasion strategies.

Also, IL18-binding protein seems to be acquired in an independent capture event from the host genome as the position of the poxvirus homologues differs markedly in MOCV and eqMCLV, underlining the importance of IL18 antagonists for the replication of molluscipoxviruses in the host epidermis. Phylogenetic analysis of the eqMCLV genome revealed a clear relationship to MOCV, however, with only 90% sequence similarity in the conserved core region it should be assigned to a new species in the genus molluscipoxvirus.
**CP 01**

Development of work management strategy for work with the causative agent of anthrax

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**Introduction:** People in the areas with anthrax problems, and who work in laboratories with anthrax are associated with a possible risk of contamination with *Bacillus anthracis* spores. A system of laboratory biological safety and biological protection should function to reduce risks. Previously, we developed analytical programs to assess the risks of infection personal based on the program of MS Office 2010. Analytical programs are based on the method of expert assessments. Programs are links for automatically determining the degrees of different risk associated.

**Objective:** Development of matrices to assess the degree of risk in contact with the causative agent of anthrax, risk of acceptance criteria and management strategy by using our proprietary analytical software.

**Materials and methods:** Patented analytical computer programs to determine the risk factors of biosecurity and biosafety, and measures to reduce the risk factors. Analytical programs consist of several modules based on the method of expert assessments. The result of each module is estimated individually. If the average security is below 50% it means that it is needed significant improvements; from 50 to 80% - needed some improvements; the indicator is over 80% - the situation is good.

**Results:** The level of biosecurity and biosecurity risks in the bacteriological laboratories of the anti-plague system was determined using analytical programs with developed matrices. The results of the analysis show that, despite the significant number of hazards when working with the causative agent of anthrax, the existing risks of biosecurity are low or acceptable (the indexes for all modules were over 80%). A plan to eliminate the existing risk has been developed. A risk management strategy has been developed. The principle of “continuous improvement” as the plan, implementation, verification, correction was recommended. After the correction and risk correction, the indexes for all modules accounted for more than 95%.

**Conclusions:** For managing of risks when working with an agent of anthrax it is useful to use the developed matrices and patented analytical programs.

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**CP 02**

The European Mobile Laboratory Consortium (EMLab) as Partner of WHO-GOARN, WHO-EDPLN and the European Medical Corps (EMC)

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The EMLab was established in the framework of IFS 2011/272- and funded by the EC: Development and Cooperation office (DevCo). The project lasted from the end of 2011 to the end of 2015 and comprised partners from Europe and Sub-Saharan African Countries. Three mobile laboratory units for diagnostics of high risk pathogens were established and a pool of scientists was trained for
deployment in case of infectious disease outbreaks. Main technical implementing partners were the Bundeswehr Institute of Microbiology in Munich, Germany (lab units and training), the Lazzaro Spallanzani National Institute for Infectious Diseases in Rome, Italy (training and focal partner NIMR Tanzania), the focal Partners in Africa, Institute for Lassa Fever Research and Control at the Irrua Specialist Teaching Hospital (ILFRC-ISTH) in Nigeria and the National Institute for Medical Research (NIMR) of Tanzania, while the project was coordinated by the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany. EMLab, under WHO-GOARN and with the input from all the partners of the consortium, like the Robert Koch Institute in Berlin, Public Health England in Porton Down, Philipps University in Marburg, INSERM-P4 Lyon, and Spiez Laboratory in Switzerland, provided molecular diagnostics in the Ebola outbreak in West Africa from March 2014 to September 2016. Since July 2016 EMLab is registered in the newly (February 2016) established European Medical Corps (EMC) as part of the voluntary pool of the Union Civil Protection mechanism and has been deployed two times since to infectious disease outbreaks in Africa, and has participated in European exercises for civil protection and humanitarian aid. In July 2016 one EMLab unit was deployed to the Yellowfever outbreak in the Democratic Republic of the Congo and in November 2017 one lab unit was deployed to the quickly contained outbreak of Marburg Virus in Uganda. In April 2017 EMLab participated at a MODEX field exercise where a lab unit was deployed to a complex disaster scenario and in May 2017 EMLab took part in a MODEX table top exercise.

EMLab’s operational readiness and maintenance is currently funded by the Global Health Protection Program of the German Federal Ministry of Health. Deployments in case of infectious disease outbreaks are funded by the German Federal Foreign Office and co-funded by the Directorate General for Civil Protection and Humanitarian Aid Operations of the European Commission (DG-ECHO).

**CP 03**

**Safe diagnostics for control of zoonotic pathogens**

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_Coxiella burnetii_ the etiological agent of Q fever causes epidemics in domestic ruminants, especially in sheep and goats. These local annual outbreaks in small ruminants are partially associated with human diseases worldwide. The often subclinical etiopathology as well as the variable sensitivity and specificity of currently used serological diagnostics prevent a fast and reliable identification of infected animals. Thus, new, innovative diagnostic tests are necessary.

The aim of the here presented project is to develop a monoclonal antibody-based pen-side test for antigen detection. For this purpose, an infection model based on trophoblasts, the natural host cells of _C. burnetii_, will be developed. By means of bacterial transcriptional analysis, strongly expressed open reading frames (ORFs) will be identified, cloned and expressed for production of recombinant proteins. These proteins will be tested for their diagnostic potential for Q fever and to exclude cross-reactions to pathogens with a similar disease manifestation in ruminants. Monoclonal antibodies against selected proteins will be produced, conjugated with fluorescence particles and used in a membrane-based heterogeneous immunoassay. The purpose is to develop a mobile readable assay with a newly constructed mobile scanner right in the field.

The development of a cheap, easy-to-use and mobile test system allows the direct and fast detection of infected animals in the field. This test will improve the detection of infected and shedding animals. The containment or elimination of these animals will limit further spreading of Q fever and may result in an improvement of human and animal health as well as reduction of economic and ecological losses.
Phenotypic and Genotypic Antimicrobial Susceptibility Testing

DP 01
Natural resistance mechanism of *Francisella tularensis* to β-lactams and a way to overcome it

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*Francisella tularensis* – the agent of dangerous human infection - is characterized by natural resistance to β-lactams (penicillins and cephalosporins). Earlier we showed that this phenomenon is formed by a complex mechanism. *F. tularensis subsp. holarctica* and *subsp. tularensis* the resistance to penicillins is associated with the production of active β-lactamase (Pavlovich N.V. et al., 1992). Under the same conditions, the enzyme in the *subsp. mediasiatica* strains is not detected, but these strains show the same resistance to the antibiotics (Tsimbalistova M.V. et al., 2014). That is why we offered a quick and easy test with nitrocefin discs for intraspecific differentiation of tularemia agent. Later V.S. Timofeev (2015) found that the mediasiatic strains produced the enzyme, but it hydrolysed the penicillins much slower. The resistance to cephalosporins is not associated with enzymatic inactivation of antibiotics, and is determined by the cell wall impermeability for drugs. As it is known, the detergents are able to increase cell permeability to various substances, including antibiotics. We investigated the effect of cationic, anionic and nonionic surfactants on the resistance of bacteria to penicillins and cephalosporins. It was established that cationic or anionic surfactants did not change the resistance to β-lactams. At the same time, *in vitro* nonionic detergents (tween 80 and triton X-100) increased sensitivity of bacteria of the three subspecies to cephalosporins (10-20 times) but not penicillins. *In vivo* experiments on the model of tularemia with white mice showed that the use of ceftazidim in combination with tween increases the effectiveness of treatment of infection caused by holarctic strains. In the case of infection with *subsp. tularensis* strains we observed prolongation of animal life for 3-4 days. Thus, for the first time in experiments *in vitro* and *in vivo* it was found that nonionic detergents increase cell wall permeability for cephalosporins and increase the sensitivity of *F. tularensis* to these antibiotics. This fact determines the prospects of further search for drugs that increase the permeability of the cell wall and increase the antibacterial activity of cephalosporins against *F. tularensis*.

DP 02
Genotypic analysis of selected antibiotic resistance patterns in *Coxiella burnetii*

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**Objective:** The obligate intracellular organism *C. burnetii* is the causative agent of the zoonotic Query (Q) fever and poses challenges to the development of novel drugs due to its intracellular life cycle. The infectious dose of *C. burnetii* can be as low as 1 to 10 single organisms, the pathogen can be distributed by wind and shows as highly environmental stable spore-like particle. Human infection usually occurs after contact with infected animals. Most of the infections are an acute disease but up to 2% can show a chronic form. Therapy is based on doxycycline, but gyrase inhibitors and macrolide antibiotics have been used also for therapy. Reported resistances against antimicrobial substances in *C. burnetii* have been scarce and antibiotic susceptibility testing has primarily been done in a pre-sequencing era and so far, this work is the first to examine various strains of *C. burnetii* for antibiotic resistance (AR) genes.

**Methods and Results:** A pipeline was written using the command language Bash and the interpreted high-level programming language Python. Via the stand-alone version of blast+, possible antibiotic resistance determining genes were located in whole genome data of *C. burnetii*. These genes were then aligned using the MAFFT algorithm. Additionally, ABRICATE was used to screen for known antibiotic resistance genes using the databases CARD, ARGANNOT and Resfinder. After analysis of genetic markers, a PCR-based single probe DNA assay was designed for a reliable genotypic identification of potential antibiotic resistances in various strains. No mobile genetic elements containing AR genes have been found. The previously annotated beta lactamase ampC of *C. burnetii* str. Dugway was found with high sequence similarity in all used genomes of *C. burnetii*. Additionally, a previously unknown mutation in the Topoisomerase IV subunit...
b gene parE harbors a polymorphism which could contribute to antimicrobial resistance against gyrase inhibitors. 

**Conclusion:** *Coxiella burnetii* does not harbor any acquired AR genes. The occurrence of beta lactamase ampC could serve as a hint for the inefficiency of beta lactams in Q fever therapy. The possible role of the parE polymorphism in the antibiotic resistance profile of *C. burnetii* still needs to be determined.

**DP 03**
WITHDRAWN

**DP 04**
Q fever – A continued threat to the military. Can antibiotic prophylaxis reduce the severity of disease?

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Q fever is caused by the intracellular bacterium *Coxiella burnetii*. It is found worldwide with the exception of New Zealand. It is a zoonotic infection with bacteria concentrated in the birth products of ruminant animals. Infection is largely transmitted via the aerosol route. It was initially identified as a military problem when thousands of people were affected during WWI. More recently Q fever has been recognised as a problem in UK troops returning from Afghanistan. Approximately 20% of patients develop Q fever fatigue syndrome which can lead to medical discharge. Doxycycline is the first line treatment with quinolones being an alternative. *C. burnetii* is classified as a CDC category B agent. There is no licensed vaccine in the UK against Q fever and therefore antibiotic prophylaxis should be evaluated.

In an initial study, A/J mice were challenged with *C. burnetii* via the aerosol route and groups treated for 7 days with a range of antibiotics starting either 1 day pre or 1 day post exposure. Weight loss, clinical signs, organ weight at necropsy and bacterial burden of the organs were measured. Doxycycline hyclate and levofloxacin administered pre and post exposure significantly protected against weight loss compared with the controls (p<0.05). Ciprofloxacin and co-trimoxazole provided no significant protection. A follow up study was performed in A/J mice focusing on an alternative preparation of doxycycline (doxycycline monohydrate). Therapy was given for either 7 or 14 days, and treatment was commenced either 1 day pre or 5 days post exposure to *C. burnetii*. The delay in post exposure therapy coincides with symptom onset in A/J mice. Both pre exposure treatment groups were significantly protected from weight loss compared to the control group (p<0.01), and weight loss was significantly reduced in both post exposure treatment groups from 24 hours after therapy was commenced (p<0.05). Both pre and post exposure treatment with doxycycline prevent dissemination of the bacteria from the lungs and spleen in contrast to the non-treated control groups. Further work is ongoing to ascertain whether a benefit exists when pre-exposure treatment is compared to early use of antibiotics at symptom onset.

**EP 01**
Vaccine Progress and New Options

Phage4Cure – a First-in-Man study with a bacteriophage product

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*Pseudomonas aeruginosa* is one of the most common bacteria found in the lungs of patients with bronchiectasis and often involved in progressive and severe course of disease. The ability of *P. aeruginosa* to rapidly acquire resistances to disinfectants and antibiotics makes it very difficult to treat and leads to the high demand for new therapies (WHO
priority 1 [critical pathogen]. Using bacteriophages (phages), viruses that specifically infect and kill bacteria is a promising alternative to antibiotics. Together, the Leibniz Institute DSMZ, Fraunhofer ITEM, Charité-Universitätsmedizin Berlin (Charité) and Charité Research Organisation initiated the joint project Phage4Cure which is funded by the German Ministry of Education and Research (BMBF). The final goal is to perform the first clinical trial with phages in Germany in close cooperation with the Federal Institute for Drugs and Medical Devices (BfArM).

Phage4Cure aims at the development of a European Medicines Agency (EMA) approvable phage based medicinal product to treat patients suffering from chronic airway infection with P. aeruginosa and the establishment of a production platform that is transferable to other phage entities with only minor adaptations in combination with a facilitated regulatory approval procedure.

The initial step is isolating suitable phages at the DSMZ that will be characterized with regard to host range, efficacy and genomic properties. Only phages will be selected for further steps that effectively lyse a large number of diverse clinical P. aeruginosa isolates and are strictly lytic. The production and purification of most promising phages according to pharmaceutical quality standards and good manufacturing practices (GMPs) for medicinal products and stability tests of the final phage investigational medicinal product (IMP) is done at Fraunhofer ITEM (Braunschweig). Phages will be combined in an inhalable product and undergo pre-clinical testing for pharmacological safety/tolerability at Fraunhofer ITEM (Hannover) and for efficacy/immunogenicity at the Charité. Following finalization of pre-clinical studies and the successful regulatory approval, this First-in-Human trial will be conducted at the research unit of Charité Research Organisation, where safety and tolerability of single and multiple doses will be investigated in healthy volunteers. Future steps involve a phase II clinical trial to evaluate dose and efficacy of this phage IMP in patients infected with P. aeruginosa.

EP 02
Three Bacillus phage receptor binding proteins specifically detect and bind to cells of Bacillus anthracis

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Bacillus anthracis, the etiological agent of anthrax disease is typically diagnosed by classical microbiological and molecular methods such as polymerase chain reaction (PCR). Alternatively, mass spectrometry techniques may aid in confirming the presence of the pathogen after culturing. However, because of the close genetic relationship between B. anthracis and other members of the B. cereus sensu lato group (such as B. cereus or B. thuringiensis) mis- or questionable identification frequently occurs in diagnostic laboratories. Bacteriophages such as phage gamma (which is highly specific for B. anthracis) have been in use for anthrax diagnostics for many decades. In this work we employed host cell-specific receptor binding proteins of (pro)phages, also known as tail or head fibers, to develop a microscopy-based approach for the facile, rapid and unambiguous detection of B. anthracis cells. For this, the genes of (putative) receptor binding proteins from Bacillus phage Wip1, Bacillus phage gamma and from the lambdoid prophage 03 located on the chromosome of B. anthracis were selected. Respective phage genes (Wip1: gp23 transcriptionally coupled with gp24; gamma: gp14) and that of lambdoid B. anthracis prophage 03 open reading frame BA4079) were PCR-amplified and cloned into an expression vector. The phage genes were heterologously expressed in Escherichia coli as C-terminal fusions with red fluorescent protein DsRed and an N-terminal Twin-Strep-tag-epitope for expression verification and purification of the recombinant proteins. Wip1 Gp23+24-protein, when expressed in E. coli strain ArcticExpress® (DE3), was soluble while BA4079- and Gamma Gp14-protein predominantly accumulated in inclusion bodies within expressing cells, had to be solubilized with 8 M urea and reconstituted by dialysis. B. anthracis cells incubated with either of the proteins was successfully surface-labelled in red. In contrast, the red fusion protein did not bind to cells of a panel of other B. cereus s.l. species or to more distantly related bacteria. Specific labelling of B. anthracis was also observed when mixed cultures with other B. cereus s.l. species were tested. From these results we anticipate that we can further develop the recombinant receptor binding proteins to become a valuable asset for the detection of B. anthracis as a confirmative and rapid means of diagnosis.

EP 03
Isolation and characterization of bacteriophages specific for multidrug-resistant Gram-negative bacteria

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The rise of multidrug-resistant gram-negative bacteria is a growing global problem which leads to the necessity of new therapies. One potential alternative for antibiotics are viruses, called bacteriophages, which can specifically lyse bacteria. In Eastern European countries bacteriophages are already used as efficient therapeutics. Regulatory issues regarding their safety and Good Manufacturing Practice (GMP) prevent their use in the EU. To address this problem, projects for establishing phage therapy like “phage4cure” and “PhagoBurn” recently obtained funding in Germany, France and Belgium. The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures collects phages on behalf of these studies as a source for host specific phage cocktails.

In this study bacteriophages isolated from surface/sewage water collected in Tunis, bacteriophages from the Millard lab specific against Escherichia, as well as ELIAVA phage preparations will be tested against a collection of 200 multidrug-resistant gram-negative bacteria containing ssp. of Enterobacter, Klebsiella and Escherichia, which were isolated from septic patients in the Tunis Military Hospital. A positive Spot Assay is followed by a Plaque Assay to isolate the phage, produce a stock and determine the exact host range. Lytic phages are going to be further characterized by Electron Microscopy and genome sequencing. Environmental phages will be trained to replicate at higher temperatures up to around 37°C, for possible use in clinical phage therapy. Combinations of phages will be tested for their sustained lytic potential against multidrug-resistant gram-negative bacteria, before being submitted to the Leibniz Institute DSMZ phage collection.

**EP 04**
**Biodiversity of bacteriophages against B. anthracis isolated in Georgia**

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Isolation and research on bacteriophages against Bacillus anthracis has a long history at the Eliava Institute of Bacteriophages, Microbiology and Virology. Several phages have been isolated in two different regions of the country. B. anthracis vaccine strains: E17, STI and 55 were used as host bacterial strains. Transmission electron microscopic (TEM) studies revealed that the most of the phages belong to Siphoviridae and only one phage to Myoviridae family. The phages are different according to phage DNA restriction and structural protein composition (by SDS-PAGE analysis). The genomic size of the phages (from 33 to 157kbp) was estimated by Pulse-Field Gel electrophoresis (PFGE). Lytic activity of phages was examined against different collections of virulent bacterial strains. According to the results, the phages reveal higher lytic activity against B. anthracis bacterial strains than to other species of the genus Bacillus.

Three bacteriophages (Siphophages) were sequenced. Genome comparisons of phages BaK1, BaK6, and BaK10 revealed significant sequence similarity to two unclassified B. cereus siphophages, Basilisk, and PBC4. Whole genome sequence comparisons (using Illumina MiSeq platform) showed high sequence similarity and significant gene content conservation between the phage genomes and the Basilisk phage. Phages BaK1 and BaK6 showed the greatest sequence similarity, while BaK10 showed closer overall similarity to phage Basilisk. Phage BaK10 was also found to uniquely encode multiple putative genes not present in Basilisk or other phages studied.

Our data indicate biodiversity of bacteriophages against B. anthracis, and provides further insight into the shared genomic architecture, host range specificity and molecular evolution of these bacterial viruses.

**EP 05**
**The Leibniz Institute DSMZ GmbH – A leading European phage bank to develop new antibacterials**

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Antimicrobial resistance (AMR) is a global threat and a cost-intensive public health problem. We must think global but may start in Western Europe to re-establish a forgotten cure that is being applied in countries of the former Soviet Union since 100 years:
bacteriophage therapy. Bacteriophages (phages) are the natural viral enemies of bacteria and exist for virtually all species. Phage therapy in clinical trials and future therapy approaches alternatively to antibiotics can become real if substantial phage diversity for prioritized pathogens is available for further purification processing and upscaling particularly for ESKAPE pathogens, the leading cause of nosocomial infections. The WHO published a priority list of bacteria (http://www.who.int/mediacentre/) in focus of new strategies to fight their distribution and AMR. The 3rd DART 2020 report (www.bundesgesundheitsministerium.de) compiles activities under six main goals to address AMR. Developing a (EU) model licensing pathway for pharmaceutical phage preparations together with the national authorities is crucial but needs virtually all species. Phage therapy in clinical trials for 30 years, the publicly funded DSMZ has had phage expertise and holds ca. 800 phages, has performed forefront research and phage studies aiming at their efficiency in practice. Researchers and clinicians should set-up phage therapy trials empowering clinically conducted therapy practice to fight AMR. Due to phage specificity high phage numbers are needed. For safeguarding them with their associated data, phage banks are required that are experienced, long-term funded, operating under SOP-driven quality management and good scientific practice. Such banks are best-located in bioresource centres (see OECD BRC Initiative at http://www.oecd.org/sti/biotech/) like e.g., the Leibniz Institute DSMZ. The mission of such service preparation time could be reduced to two or even one single visit in the future. We'll present new preliminary data of 170 subjects from a third RCT on intradermal rabies vaccination, evaluating the added value of topical imiquimod and/or an intradermal device to enhance immunological responses to rabies vaccine. ; Conclusion: The immunity provided by the shortened ID series is immunogenic and robust and should be considered as an investment once in a lifetime in operational troops. Shortened rabies vaccine schedules are very promising and the preparation time could be reduced to two or even one single visit in the future.

EP 07
An investigation of protective immunogenic proteins of Acinetobacter baumannii with immunoproteomic analysis

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EP 06
Rabies immune responses by shortened intradermal rabies preexposure vaccination schedules in Belgian Armed Forces

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Background: Rabies causes almost invariably fatal encephalitis. Low-dose Intradermal (ID) Pre-exposure Vaccination Schedules has been proven economical, safe, immunogenic and long-lasting. ; Objective: We will present the data on initial Neutralising Antibody Response after Primary Vaccination after different ID Rabies Pre-exposure Vaccination Schedules in the BE Armed Forces. ; Method: Neutralizing Antibody Titers against rabies virus were evaluated with the Rapid Fluorescent Focus Inhibition Test. A titer ≥ 0,5 IU/ml is considered to be boostable life-long. Results: We will present our data of six different studies in Belgian military troops. More than 10.000 soldiers are intradermally vaccinated against rabies in the Belgian Army. In three large retrospective studies of 881, 500, and 2000 Belgian soldiers, a sufficient initial antibody response of ≥ 0,5 IU/ml over years for two different rabies vaccination schedules (first study: day 0, 7, 28, 365 and second and third study: day 0, 7, 28) was shown in 100%, 82% and 100% of subjects respectively. Recent data of two prospective randomized clinical trials (in 500 and 303 Belgian soldiers) show that 100% and 81,5% of subjects respectively had a sufficient initial antibody response of ≥ 0,5 IU/ml supporting the idea that the preparation time for alternative pre-exposure schedules could be reduced from 28 days to 7 days and further to 1 day. We'll present new preliminary data of 170 subjects from a third RCT on intradermal rabies vaccination, evaluating the added value of topical imiquimod and/or an intradermal device to enhance immunological responses to rabies vaccine. ; Conclusion: The immunity provided by the shortened ID series is immunogenic and robust and should be considered as an investment once in a lifetime in operational troops. Shortened rabies vaccine schedules are very promising and the preparation time could be reduced to two or even one single visit in the future.
Introduction and Aim: Multidrug resistant *Acinetobacter baumannii* (MDRAB) is one of the most important biothreat agents for the patients especially with war wounded and/or caring intensive care units. Currently, antibiotic choices are very limited in MDRAB infection and vaccine is not available. The aim of this study was to investigate the protective immunogenic proteins and potential vaccine candidates for *A. baumannii* infection.

Materials and Methods: For immunoproteomics analysis of *A. baumannii*, standard strains ATCC 17978 (sensitive), ATCC BAA-1710 (multidrug-resistant) and two clinical isolates were used in the study. Serum specimens were collected from 29 patients with bacteremia due to *A. baumannii*. Protein samples of outer-membrane and secretome were prepared. Two-dimensional gel based immunoblot analysis for pre-screening of proteins was performed. Then, *A. baumannii* specific protein reacting patient IgGs, isolated with protein-G labeled micro-beads carrying human IgG specific antibody, were separated by immunopolludown. After mass spectrometry (MS) analyses of *A. baumannii* proteins bound to patient IgGs isolated by immunoprecipitation were identified by using Protein Pilot and MASCOT software. Bioinformatic analyses were carried out by Immune Epitope Data-Base.

Results: 280 proteins which were reacting with patient’s sera were detected in 29 patients with bacteremia. 38 of these proteins showed strong antigenic properties. All of these proteins were belong to resistant standard *A. baumannii* strain. These numbers have been reduced with bioinformatics tools from 38 to 9 antigenic candidates for cloning and expression according to the criteria of solubility, transmembrane property and B lymphocyte-specific epitopic region characteristics. The expression studies will be carried-out with a future project.

Conclusion: Further studies are needed to use these antigenic structures of *A. baumannii* as vaccine candidates or diagnostic tools.

**EP 08**

Development of a heat-stable MVA-based prime/boost vaccine against chronic hepatitis B

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A major challenge in developing effective therapeutic vaccination against chronic hepatitis B is the induction of neutralizing antibodies and virus-specific effector T cell responses. Therefore we established a heterologous protein prime/MVA vector boost therapeutic vaccination scheme that has been proven to break immune tolerance in mouse models of chronic hepatitis B. Vaccine components, however, suffer from thermal instability and require appropriate cooling chains during transportation and storage. This is a general issue that has been discerned by the WHO and severely limits the distribution and use of vaccines in low-income countries and remote areas. In this study, we therefore developed both, a thermostable lyophilized hepatitis B virus (HBV) core antigen (HBcAg) and HBV-core expressing MVA vector (MVA-HBc). Thermostability of protein and viral vector components was achieved using the Stabilizing and Protecting Solutions (SPS®) formulation technology platform.

36 SPS®-formulations were tested for their stabilizing effect on HBcAg, MVA-HBc and cell models compatibility. Infectivity of the viral vector was determined by TCID50 assay, while molecular integrity of HBcAg was analyzed by native agarose gel electrophoresis (NAGE) and dynamic light scattering (DLS). Morphological analysis was performed for both components by transmission electron microscopy (TEM). Immunogenicity and safety of vaccine components stabilized by optimized SPS®-formulations were validated in C57/BL6 wildtype and HBV-transgenic mice (HBV 1.3.32).

SPS®-formulated vaccine antigens and vectors remained morphologically stable and maintained their functionality, infectivity and integrity even after 45 °C temperature challenge for up to four weeks. In vivo experiments with SPS®-formulated thermostable vaccines were well tolerated and induced HBV-specific antibodies, Th1/Th2, CD4 -and an effector CD8 T cell response in spleen and liver of HBV-transgenic mice. Consequently, the stabilized therapeutic vaccine - even after stressing - leads to a downregulation of all HBV-markers thereby breaking immune tolerance.

In conclusion, the heterologous protein prime/MVA-boost vaccination strategy comprising SPS®-formulated thermostable components may enable the commercialization of highly functional and heat-stable vaccines to treat chronic hepatitis B virus infection worldwide.

**EP 09**

Identification of candidate *Coxiella burnetii* T-cell epitopes for a novel human Q fever vaccine

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Coxiella burnetii, the causative agent of Q fever, is a Gram-negative intracellular bacterium transmitted via aerosol. Regulatory approval of the Australian whole-cell vaccine Q-VAX® in the US and Europe is hindered by reactogenicity in previously exposed individuals. The aim of this study was to identify and rationally select C. burnetii epitopes for design of a safe, effective and less reactogenic T-cell targeted human Q fever vaccine.

Immunoinformatic methods were used to predict 65 HLA class I and 50 HLA class II C. burnetii epitopes. HLA binding assays confirmed 89% of class I and 75% of class II predictions, and 11 HLA class II epitopes elicited IFN-γ responses following heterologous DNA/DNA/peptide/peptide prime-boost immunizations of HLA-DR3 transgenic mice. Human immune responses to the predicted epitopes were characterized in individuals naturally exposed to C. burnetii during the 2007-2010 Dutch Q fever outbreak. Subjects were divided into three groups: controls with no immunological evidence of previous infection and individuals with responses to heat-killed C. burnetii in the Q-detect™ whole blood IFN-γ release assay (IGRA) who remained asymptomatic or experienced clinical Q fever during the outbreak. Recall responses to C. burnetii epitopes were assessed by cultured IFN-γ ELISpot. While HLA class I epitope responses were sparse in this cohort, we identified 21 HLA class II epitopes that recalled T-cell IFN-γ responses in 10-28% of IGRA+ subjects. IGRA+ individuals with past asymptomatic and symptomatic C. burnetii infection showed a comparable response pattern and cumulative peptide response which correlated with IGRA responses. None of the peptides elicited reactogenicity in a C. burnetii exposure-primed guinea pig model.

These data demonstrate that a substantial proportion of immunoinformatically identified HLA class II epitopes show long-lived immunoreactivity in naturally infected individuals, making them desirable candidates for a novel epitope-based Q fever vaccine.

**EP 10**

Evaluation of humoral immunity and genomic studies of vaccine strains for improvement of smallpox preparedness in Brazil

Smallpox was eradicated in 1980 thanks to worldwide vaccination with vaccinia virus (VACV). However, variola virus still represents a risk as a bioweapon. In this regard, some governments have developed strategies for preparedness, such as first-responder vaccination and the maintenance of smallpox vaccine stockpiles. In Brazil smallpox vaccination ceased in the early 1980s. To contribute with the smallpox preparedness in Brazil, we have assessed the smallpox immune status of a risk group comprising Brazilian army militaries trained to combat Chemical, Biological, Nuclear or Radiological (CBRN) threats. In this study, we investigated the presence of total and neutralizing anti-VACV antibodies in sera from a cohort of 257 military volunteers (16 women and 241 men). Requested information included age, self-declaration of smallpox vaccination in childhood, presence of vaccination “take” in the arm, and history of direct contact with cattle. It is noteworthy that Cantagalo virus is an endemic VACV strain in Brazil that causes zoonotic infections in dairy cattle. To analyze total anti-VACV IgG, we standardized a colorimetric ELISA test, using purified VACV strain WR as the antigen. Of the total population, 9.7% (25) tested positive, 5.5% (14) were considered borderline and 84.8% (218) tested negative. Except for one 25 yo positive individual, all positive and borderline volunteers were ≥38 yo, probably due to vaccination in childhood. That 25 yo positive military reported previous contact with cattle followed by the development of pustular lesions on the hands. The presence of neutralizing antibodies in positive and borderline sera was investigated by PRNT50. Of the 25 ELISA positive sera, only 4 (1.6% of total participants) presented protective titers (≥1/32), being two highly protective (≥1/60). As part of our efforts, we have also investigated the smallpox vaccine strain Wyeth manufactured in 1972. It is currently unknown whether the Wyeth strain was included in the manufacture of the main Brazilian vaccine VACV-IOC. Therefore, we sequenced the whole genome of three clonal isolates of VACV Wyeth, using the NGS platform Illumina HiSeq 2500. Contig assembly generated genomes of 200,375 bp, 198,436 bp and 198,512 bp, with >2060x coverage. The phylogenetic inference shows that all clones branch with clones of the American
vaccine Dryvax, but not with VACV-IOC. This is an on-going study that aims to provide guidance the Brazilian smallpox preparedness program.

EP 11
The *Yersinia pestis* transaldolase is a component of antigenic complex

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**Introduction:** The immunochemical determination of the plague agent *Yersinia pestis* is based on the detection of capsular antigen CaP1. The loss of the ability to synthesize the protein leads to the evasion of the *Y. pestis* bacteria from detection by immunological tests [Drozdov I.G. et al. 1993, Friedlander A.M. et al., 1995]. Fraction V is another diagnostic complex that was suggested for the detection of *Y. pestis* strains [Bozhko N.V. et al., 1998, 2006]. It could be used for diagnostic purposes, but its component composition was not identified. The purpose of this study is to determine the immunologically active components of the FV antigen.

**Materials and methods:** The FV preparation was obtained from pFra- and pCad- strain *Y. pestis* Otten 106 by the method proposed by Bozhko N.V. (2006). Investigation of the FV-components was carried out using two-dimensional (2D) electrophoresis, immunoblotting (Western blot) and MALDI-TOF-mass spectrometry. Western blot was performed using monoclonal antibodies E6H8 which have specific activity against FV. PCR copy of the transaldolase gene was cloned into vector plasmid pGEM-T with the help of pGEM-T Easy Vector Systems (Promega).

**Results:** Visualization of the FV components by 2D electrophoresis and Western blot allowed to reveal several proteins which interacted with monoclonal antibodies against FV. The largest signal was obtained from the protein which migrated at the level of 35 kDa and pI 5.3 in the 2D gel. The mass spectrometry of the protein extracted from the gel showed that it can be identified as transaldolase. The *Y. pestis* transaldolase gene was amplified by PCR and cloned into the vector plasmid pGEM-T. The resulting recombinant E. coli strain which carried the plasmid containing the transaldolase gene reacted with the horse antiplaque serum in the gel precipitation reaction. The control strain containing only the vector plasmid pGEM®-T didn’t react with the antiplague serum.

**Conclusions:** The results of the present study showed that *Y. pestis* transaldolase has pronounced immunological activity. The recombinant strain can be used for the purification of *Y. pestis* transaldolase which is perspective for the development of the diagnostic and prophylactic preparations.

EP 12
Biodistribution of the vaccine candidate MVA-MERS-S after single dose intramuscular inoculation in mice

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Modified Vaccinia Virus Ankara (MVA) is a highly attenuated and replication-deficient virus, serving as well-established vector vaccine against infectious diseases. In previous clinical evaluations, many recombinant MVA vaccines have shown an excellent safety profile, suggesting the possibility of safe MVA-based immunizations even in immunocompromised individuals and in persons with severe comorbidities. Still, it is highly desirable to collect data concerning the non-clinical safety of a new candidate MVA vector vaccine. Here, we assessed the biodistribution of a recombinant MVA vector vaccine expressing the full-length spike protein of Middle Eastern Respiratory Syndrome (MVA-MERS-S) in mice after a single dose intramuscular inoculation. In the same manner, control mice were inoculated with a well-characterized control recombinant virus MVA-GFP-mCherry and PBS. The biodistribution and lesion profile was monitored at different time points by histology, immunohistochemistry and real-time PCR.

Both recombinant MVA viruses produced similar lesions at the inoculation site. Gross lesions at the parenteral site included mild edema and swelling and hyperplasia of the draining lymph nodes. Histology of the parenteral site confirmed edema and inflammation with concurrent focal myonecrosis. The draining lymph nodes displayed increased paracortical cellularity. Inoculation of MVA did not lead to detectable lesions in in tissues other than the parenteral site. Both MVA and recombinant antigen were detected only at the site of inoculation in mesenchymal cells. Real-time PCR analysis of >240 tissue samples detected MVA-DNA predominantly at the injection site and in the draining lymph nodes. A single intramuscular inoculation of MVA-MERS-S does not lead to histologically or immunohistochem-
ically detectable lesions in tissues peripheral to the parenteral site. The lesions and antigen distribution pattern are indistinguishable from those found after use of the control recombinant MVA-GFP-mCherry. Levels of inflammation and the hyperplasia of draining lymph nodes were considered in line with the expected immunological response to the vaccine inoculation. Real-time PCR results suggested continuous clearance of the candidate vaccine during the observation period.

**EP 13**

**Generation and production of high-titer reporter HBV for monitoring infection and single-cell selection**

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Recombinant Hepatitis B Virus (rHBV) expressing transgenes allow monitoring of infection and selection of infected cells. Due to the compact genome organization and size restriction, the transgenes must be inserted into the open reading frame of essential HBV genes. Hereby, the surface protein genes proved a suitable region for replacement. Since this overlaps with the viral polymerase, trans-complementation of both lacking viral proteins is needed to allow efficient packaging of rHBV genomes and particle genesis. This step is usually accomplished by cost- and labor-intensive co-transfections, which often results in low viral titer and variable quality of virus stocks, limiting the broad applicability of rHBV.

Therefore, we generated a stable trans-complementation cell-line, expressing HBV polymerase and surface proteins. Based on this cell-line, we generated various stable cell-lines continuously secreting different rHBV (e.g. Luciferase, Zeocin, DTR, tRFP, etc.) into the supernatant. We optimized the production and purification process, ensuring high-titers (up to 1e10 GE/mL) of rHBV and thereby facilitating different experimental setups.

Using rHBV expressing reporter genes, we can monitor infection cost-efficiently, quantitatively and with high-sensitivity. Furthermore, rHBV expressing selection markers allow us to perform positive and negative selection of infected cells. Most importantly, rHBV expressing fluorescent proteins enables us to investigate single-cell infections via confocal microscopy, to identify zones of high and low infection, and single-cell sorting (up to 47% positive cells, with sequential infection) allows cell-specific genome or proteome analysis.

Against our expectation, after reseeding of sorted rHBV infected HepG2-NTCP cells, we did observe clonal expansion of cells with continual expression of transgenes, indicating integration of rHBV into the host cell genome and offering an option to study this.

**EP 14**

**Development of a vaccine for Lassa fever using Modified Vaccinia Ankara virus as a vector**

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Lassa fever remains the most imported viral haemorrhagic fever in Europe and is responsible for 5000 deaths per year throughout Western Africa. There is no vaccine and treatment is often ineffective. Public Health England (PHE) has previously demonstrated success with Modified Vaccinia Ankara (MVA) vaccines expressing proteins to viruses such as Crimean-Congo Haemorrhagic Fever (CCHF), and as such, intend to widen the scope by developing constructs with Lassa virus antigens. This study investigated the immunogenicity (in mice) and efficacy (in guinea pigs) of a MVA-Lassa nucleoprotein (MVA-LassaVaccNP) vaccine as a prime/boost or single vaccination regime. ELISA and ELISpot assays confirmed humoral and T-cell immunity in both a prime/boost and prime vaccination with the prime/boost regime observing a statistically increased response compared to a prime only vaccine (P<0.0001). The vaccine also offered protection against disease manifestations in guinea pigs subsequently challenged with virulent Lassa virus. Clinical symptoms, weight loss and temperature increases were observed in all control animals. In comparison, no clinical symptoms, fever or weight loss was observed in any of the vaccinated animals suggesting either a single or prime/boost regime confers protection.

In conclusion, the MVA-LassaVaccNP construct developed in this project elicits an immune response in mice, demonstrates efficacy against Lassa virus disease in guinea pigs and is suitable for further preclinical and clinical development.
New Approaches to Antiviral and Antibiotic Drug Discovery

GP 01
Investigation of new substances – Antiviral activity and determination of their possible application for virus infections eradication

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Introduction: The viral diseases are still increasing in the world. Especially dangerous are new unknown diseases because their treatment and prevention has not been developed. Due to rapid emergent infections flow, funds are needed to deal with them, which immediately will be able to reduce or stop the pathogens activity. But universal antiviral means till now have been absent. The investigations in this field are aimed to finding new approaches of the infections eradication. We conducted a study of antiviral properties of new 50 indole-containing condensed tetracyclic compounds in preclinical study that were synthesized by S. Dgebuadse.

Methods: We used the members of Herpesviridae and Coronaviridae Families (animal pathogens) with high infectious titers; continuous animal cell cultures: – cell culture of versenised swine embryonic kidney, BHK-21, Vero, SK-6. For investigation of anti-virus properties we used a complex of standard methods in vitro and in vivo systems (more than 4,000 experiments with triple repetitions). We investigated five different schemes of tested substances antiviruses actions in creation of treatment by viral infection process, as preventive as treatment. Also we determined the acute and subacute toxicity of promising indole derivatives.

Results: We were shown the data about examined compounds that significantly reduced the infection activity of tested virus strains in vitro systems in different ways. But the highest antiviral activity among tested substances was shown by decreased viral infection activity to 7.04±0.04 lg TCID50/ml. One compound had a significant antiviral effect as on DNA as on RNA viruses (in all schemes that allow to recommend it for the creation of a universal antiviral drug). 18 found indole-containing condensed tetracyclic compounds able to reduce simultaneously titer of both virus Families members in certain scheme. Among them - 12 inhibited of virus activity on 2.47±0.06 – 5.38±0. TTSD50 0.9 lg / cm3 (P <0.05), indicating a broad spectrum of action of these compounds.

Conclusion: We have identified substances that will be suitable in clinical research for testing in eradication virus infections. The results were obtained through STCU grant # p450 by financial support of KCP (USA).

GP 02
Biological evaluation of antiviral agents versus encephalitis viruses using live cell microscopy

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The goal of this project is to establish a complex infection model for the blood-brain-barrier for the evaluation of antivirals versus viruses causing encephalitis. Initially measles and vaccinia viruses were selected, as GFP reporter viruses are available and experiments can be done under S2 conditions. Measles virus (Paramyxoviridae) is a highly infectious human pathogen that can lead to high fever, pneumonitis and conjunctivitis, as well as the typical meases rash. Use of the efficient live-attenuated vaccine has recently lapsed in developed countries leading to small and medium scale outbreaks. While vaccination is safe, wildtype measles infection can lead to potentially fatal medium and late term complications (e.g. measles/subacute inclusion body encephalitis and SSPE). Vaccinia virus (Orthopoxviridae) was used for the eradication of variola virus in 1980. Vaccination can lead to severe complications including postvaccinal encephalitis. Effective small molecule antivirals would be useful to treat manifest cases and complicated infections/vaccinations.

The project will establish live-cell microscopy of single and multiple cell infections (glia, endothelial and myeloid cells) with reporter viruses in preparation for the testing of the µ-Slide Membrane ibiPore Flow system as a model of the blood brain barrier (BBB). GFP-expressing measles virus IC323 and vaccinia virus v300/354 will be used to analyze infection kinetics in live cells. The ibidiBOX live
Coxiella burnetii, the causative agent of Q fever, is a highly infectious pathogen that has been classified as a category B biological weapon agent. Effective antibiotic treatment is available for the acute phase of the illness, however treatment for the chronic form remains difficult. Moreover, the increase in antibacterial resistance found in other bacterial pathogens highlights the need for alternative therapeutics to treat Q fever. We developed a group of small molecule inhibitors against the macrophage infectivity potentiator (Mip) protein, an immunophilin that has been shown to be important for survival and pathogenesis of a number of Gram-negative intracellular pathogens. Since immunophilins typically exhibit peptidylprolyl cis-trans isomerase (PPIase) activity, the ability of these compounds to inhibit the PPIase activity of recombinant C. burnetii Mip was determined using a protease-coupled assay. We found that these compounds inhibit the enzymatic activity of recombinant Mip. Importantly, following infection of THP-1 cells (a human macrophage-like cell line) with Nine Mile Phase II C. burnetii, intracellular replication was reduced in the presence of Mip inhibitors over five days of intracellular infection. This suggests that these Mip inhibitors may serve as a novel antivirulence drug for therapeutic use against C. burnetii.

**GP 04**

**Biological evaluation of novel small-molecule antiviral agents versus Chikungunya virus**

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus causing flu-like illness with severe long-lasting arthralgia and therapy-resistant headaches, but only rarely meningoencephalitis, in the human host. There have been several significant outbreaks in recent years. So far, there are no CHIKV specific vaccines or therapeutics. Starting from the structures of antiviral hits that have previously been identified with in silico techniques (Basetto et al., 2013, Tardugno et al., 2018), 34 novel structural analogues have been designed and prepared. Activity versus CHIKV was tested in simian VeroB4 cells, where CHIKV causes cytopathogenic effects (CPE), but only limited cell death, presumably due to viral gene products inhibiting apoptosis (Joubert et al., 2012). CHIKV does not replicate in A549, dbtreg or HUH7 cells, but causes a distinct CPE in U138 glioblastoma cells.

An initial ranging viability assay at 10µM revealed at least one compound with IC50 ≤ 10µM and little toxicity in U138 as well as in VeroB4 cells. Further biological evaluation of these compounds in different cell lines (VeroB4, Huh7, U138, U254 and U87), characterization of lead compounds in cells of CNS origin and different assay methods (plaque assay/xCELLigence) are ongoing and will be reported. This work provides the foundation for further investigation of promising novel structures as antiviral agents against Chikungunya virus.

**GP 05**

**Flaviviral NS4A induces autophagy in human epithelial cells**

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Infection of epithelial cells with flaviviruses often does not lead to cell death. This has been linked to induction of autophagy by non-structural protein NS4A of Dengue-2 and Modoc viruses. Prevention of cell death leads to prolonged replication of these viruses in epithelial cells and fibroblasts (McLean et al., 2011).

Here, we report that in epithelial cells, overexpression of NS4A from three different flaviviruses (Zika, Tick-borne encephalitis virus (TBEV), Yellow fever virus (YFV)) leads to the upregulation of autophagy marker LC3B-II. Epithelial cells were transfected with expression vector pIRESneo encoding NS4A from Zika, TBEV and YFV and lysates were collected after 48 h. Elevated levels of LC3B-II were detected using quantitative western blot analysis and immunofluorescence. Preliminary caspase 3/7 data indicate that flaviviral NS4A induced autophagy reduces level of apoptosis in epithelial cells after treatment with staurosporin.

The observed levels of autophagy induction were low, which may be due to viral cofactors being involved in the upregulation of autophagy. To confirm this hypothesis further experiments using wildtype flavivirus infection of susceptible cell lines are planned.

Our observations support previous hypotheses that NS4A plays an important role in flavivirus replication due to upregulation of autophagy and down-regulation of apoptosis in infected cells. Due to that, NS4A might be a useful target for antiviral substances.

**GP 06**  
Biological evaluation of a novel cell targeting L-ddBCNA compound against measles virus

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Measles virus wild-type strain (MeV WT) is the aetiological agent of measles, a highly contagious disease which contributed to 90,000 deaths globally in 2016. However, no effective anti-measles therapeutics are currently available. Here we report mechanism of action (MoA) studies performed for cf2642, a novel cell targeting [L]-chiral dideoxy bicyclic pyrimidine nucleoside analogue (L-ddBCNA) that we previously showed to be active against measles and vaccinia viruses. Vero cells expressing the MeV-WT receptor SLAM (signalling lymphocyte-activation molecule) were used for all studies.

Western blot analysis of LC3-II (a protein marker for a cellular process called autophagy) levels 12 hrs post-treatment with 10µM cf2642 showed inhibition of MeV-WT induced autophagy. MeV-WT has been previously reported to cause a transient increase in uptake of fluorescently labelled dextran in VerohSLAM cells. This was attributed to virus entry occurring via an endocytic pathway called macropinocytosis. Here we have shown using similar conditions that MeV-WT causes a 46% increase in uptake of fluorescently labelled dextran at 15 min post-administration of virus and this was reduced to below basal levels with 10µM cf2642. MeV-WT infection of susceptible cells results in the clustering of SLAM receptors, hypothesized to aid virus entry. The early effects of cf2642 against MeV-WT were studied using SLAM-clustering antibodies, thereby mimicking virus-receptor interaction in a non-infectious setting. Confocal microscopy analysis showed a 40% decrease in clustering-induced SLAM internalisation after 90 min treatment with 10µM cf2642. A time of addition assay using EGFP expressing MeV-WT strain (MeV EGFP) showed a 58% reduction in EGFP positive cells even when cf2642 was added 12hrs post infection, indicating that a cellular target important during the entire virus life cycle was likely to have been affected. A virus-entry/spread assay done to elucidate the MoA of cf2642 indicated that the drug most likely imparts its anti-measles activity by affecting virus spread and partially inhibiting virus entry. We propose that cf2642 exhibits anti-measles activity via two mechanisms involving membrane traffic that are known to be utilised by the virus. Whether these effects on autophagy and cell entry (macropinocytosis) are related remains to be determined. The results provide insights into the potential use of this drug as a cell-targeting antiviral and autophagy inhibitor.

**GP 07**  
Antiviral resistance and multi-drug-resistance in herpes virus infections (HSV and HCMV)

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Antibiotic and multi-drug-resistance is well-
described since about 60 years and antiviral resistance/multi-drug-resistance is described since about 40 years. In herpes virus infection, especially in high risk patients such as stem cell transplant recipients, solid organ transplant recipients and even in newborns it is an emerging clinical problem. The development of antiviral resistance in herpes virus infection requires drug-exposure. In treatment naive patients this phenomenon is not existing. This is in clear contrast to antiviral resistance in the HIV or influenza field. The mutations mediating antiviral resistance in HSV/ or HCMV-infections are located either in a kinase gene or in the polymerase gene. For the detection of the antiviral resistance genotype tests were developed in addition to the time and labour consuming phenotype testing based on viral isolates from cell culture.

We developed and modified both test systems, including the marker transfer analysis. There is an urgent need for marker transfer analysis in cases of newly discovered mutations in the two relevant genes mediating resistance. In all mentioned genes, there are polymorphisms described. The categorization of a new mutation in the HSV thymidine kinase gene (UL23) or the HSV polymerase gene (UL30) as well as HCMV serin-threonine kinase gene (UL97) or the HCMV polymerase gene (UL54) is not possible without marker transfer analysis!

We have identified and characterized a number of new mutations mediating antiviral resistance or even multi-drug-resistance in immunosuppressed patients in the transplant setting. In addition, we found in the last few years antiviral drug-resistance in HCMV congenitally infected newborns. In respiratory syncytial virus (RSV) infection there are new treatment options for prophylaxis of severe respiratory tract infection in high risk infants. Close to 10% of the treated infants so far developed resistance. This new phenomenon is associated with mutations in the F-gene region of RSV. We developed a marker transfer test system for this and a culture-based drug-susceptibility test.

In virology, the problem of antiviral resistance and multi-drug-resistance never reached the dimension of the problem of multi-drug-resistance in antibiotics. The reasons for this fundamental discrepancy will be discussed focussing on the stringency and the compliance of guidelines, the developed applied methods and overall concepts.

**GP 08**

**Lipid A remodeling is a patho-adaptive mechanism that impacts lipopolysaccharide recognition and intracellular survival of Burkholderia pseudomallei**

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Burkholderia pseudomallei causes the severe disease melioidosis. The bacterium subverts the host immune system, replicates inside cells, and host mortality primarily results from sepsis related complications. Lipopolysaccharide (LPS) is a major virulence factor and mediator of sepsis that many pathogens capable of intracellular growth modify to reduce their immunological “foot print”. The binding strength of B. pseudomallei LPS for human LPS binding protein (hLBP) was measured using surface plasmon resonance. The structures of lipid A isolated from B. pseudomallei under different temperatures were analyzed by MALDI-TOF and the gene expression of two lipid A remodeling genes, lpxO and pagL, were investigated. The LPS were characterized in their ability to trigger TNF-α release and activate caspase-11 triggered pyroptosis by introduction of LPS into the cytosol. Lipid A from long-term chronic isolates was isolated and characterized by MALDI-TOF and also by ability to trigger caspase-11 mediated cell death. Lipid A from B. pseudomallei 1026b lpxO and pagL mutants was characterized by positive and negative mode MALDI-TOF to ultimately identify their role in lipid A structural modifications. Replication of lpxO and pagL mutants and their complements within macrophages showed that lipid A remodeling can effect growth in host-cells and activation of caspase-11 mediated cytotoxicity.
**IP 01**
Constructions and evaluation of Chikungunya virus pseudotyped virus for neutralization assays

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Chikungunya virus (CHIKV) with a single stranded positive-sense RNA genome, belongs to Alphavirus genus of Togaviridae family. Its infection mainly causes abrupt high fever, rashes, headache, and especially severe joint pain that can last for several months or years. CHIKV, a mosquito-borne arbovirus, is considered to be an emerging/re-emerging pathogen that becomes one of the most important global health concerns due to rapid increase in epidemics. There is no currently available vaccine or antiviral against CHIKV. Due to the limitation of handling CHIKV at Biosafety Level 3 (BSL-3) facilities, we set to generate CHIKV glycoproteins pseudotyped virus (CHIKVpseudo) using lentiviral vector systems. In this study, we firstly identified the structure protein sequence of a CHIKV strain isolated in Korea (KNIH/2009/77). Based on this sequence information, we constructed a lentiviral vector-based pseudotyped virus expressing the structural proteins of CHIKV. We then examined potential application of CHIKVpseudo for neutralization assays. IC₅₀ values of neutralizing assays with CHIKVpseudo were similar to those of plaque reduction neutralization assays using CHIKV, suggesting a useful and safe method to test the neutralizing activity of anti-sera against CHIKV by using CHIKV pseudotyped virus.

**IP 02**
Intra- and interspecies differentiation of the pathogenic *Yersinia* by PCR analysis of the siderophore pestibactin biosynthesis genes

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Plague causative agent *Yersinia pestis* is known to produce siderophore yersiniabactin (Ybt) encoded by the high pathogenicity island widely distributed between pathogenic *Enterobacteria*. Our study revealed that *Y. pestis* ssp *pestis* is able to produce a more specific siderophore pestibactin (Pbt) which plays a role of an iron-dependent regulator of the siderophore activity, autoagglutination and virulence of *Y. pestis* [Podladchikova et al., 2015]. Pbt was not found in endemic *Y. pestis* (spp altaica, liissarica, ulegeica, talassica, microtus), *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* strains. Investigation of Pbt structure revealed that it represents an aberrant form of *Y. pseudotuberculosis* pseudochelin siderophore encoded in the *ynp* locus [Rakin et al., 2012]. Analysis of this locus and evaluation of its possible application for intra- and interspecies differentiation of pathogenic *Yersinia* were the subject of the present research. Bioinformatic study of the *ynp* locus in *Yersinia* strain, the whole genome sequences of which are available in NCBI (31 *Y. pestis*, 11 *Y. pseudotuberculosis*, 10 *Y. enterocolitica* strains), showed that the locus structure varied in different *Yersinia* species. *Y. pestis* ssp *caucasicus* and *Y. enterocolitica* strains lacked the entire *ynp* locus which suggests it is the reason of Pbt absence in those strains. *Y. pestis* and *Y. pseudotuberculosis* strains have different structures of genes that code for two multi-module nonribosomal peptide/polyketide synthetases partially homologous to *irp1* and *irp2* Ybt biosynthesis genes. The *irp2*-like gene carries insertions in the endemic *Y. pestis* strains and various length deletions in different *Y. pseudotuberculosis* strains. The *irp1*-like gene has an IS100 insertion in *Y. pestis* ssp *pestis* strains, but not in endemic *Y. pestis* and *Y. pseudotuberculosis* strains. Different primers for the PCR analysis of *irp1* - and *irp2*-like genes were constructed and used for the analysis of 20 *Y. pestis* ssp *pestis*, 15 endemic *Y. pestis* and 30 *Y. pseudotuberculosis* strains of 15 serotypes. The analysis showed that the primers allowed to differentiate *Y. pestis* ssp *pestis* from endemic *Y. pestis* strains, as well as to distinguish them from *Y. pseudotuberculosis*.

Thus, the results indicate that PCR with primers complementary to the variable regions of *irp1*-like and *irp2*-like genes could be used for the intra- and interspecies differentiation of pathogenic *Yersinia*.

**IP 03**
The 16S ribosomal RNA genes of *Bacillus anthracis* and their transcripts can be specifically detected by Fluorescence in situ hybridization and digital PCR

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Plague causative agent *Yersinia pestis* is known to produce siderophore yersiniabactin (Ybt) encoded by the high pathogenicity island widely distributed between pathogenic *Enterobacteria*. Our study revealed that *Y. pestis* ssp *pestis* is able to produce a more specific siderophore pestibactin (Pbt) which plays a role of an iron-dependent regulator of the siderophore activity, autoagglutination and virulence of *Y. pestis* [Podladchikova et al., 2015]. Pbt was not found in endemic *Y. pestis* (spp altaica, liissarica, ulegeica, talassica, microtus), *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* strains. Investigation of Pbt structure revealed that it represents an aberrant form of *Y. pseudotuberculosis* pseudochelin siderophore encoded in the *ynp* locus [Rakin et al., 2012]. Analysis of this locus and evaluation of its possible application for intra- and interspecies differentiation of pathogenic *Yersinia* were the subject of the present research. Bioinformatic study of the *ynp* locus in *Yersinia* strain, the whole genome sequences of which are available in NCBI (31 *Y. pestis*, 11 *Y. pseudotuberculosis*, 10 *Y. enterocolitica* strains), showed that the locus structure varied in different *Yersinia* species. *Y. pestis* ssp *caucasicus* and *Y. enterocolitica* strains lacked the entire *ynp* locus which suggests it is the reason of Pbt absence in those strains. *Y. pestis* and *Y. pseudotuberculosis* strains have different structures of genes that code for two multi-module nonribosomal peptide/polyketide synthetases partially homologous to *irp1* and *irp2* Ybt biosynthesis genes. The *irp2*-like gene carries insertions in the endemic *Y. pestis* strains and various length deletions in different *Y. pseudotuberculosis* strains. The *irp1*-like gene has an IS100 insertion in *Y. pestis* ssp *pestis* strains, but not in endemic *Y. pestis* and *Y. pseudotuberculosis* strains. Different primers for the PCR analysis of *irp1* - and *irp2*-like genes were constructed and used for the analysis of 20 *Y. pestis* ssp *pestis*, 15 endemic *Y. pestis* and 30 *Y. pseudotuberculosis* strains of 15 serotypes. The analysis showed that the primers allowed to differentiate *Y. pestis* ssp *pestis* from endemic *Y. pestis* strains, as well as to distinguish them from *Y. pseudotuberculosis*.

Thus, the results indicate that PCR with primers complementary to the variable regions of *irp1*-like and *irp2*-like genes could be used for the intra- and interspecies differentiation of pathogenic *Yersinia*. 
For many years the 16S ribosomal RNA (rRNA) genes of *Bacillus anthracis* have been considered a poor target for discriminatory detection of the pathogen and diagnosis of anthrax disease. This is because within the *B. cereus* sensu lato group, of which *B. anthracis* is a member, most species have but identical gene sequences. Recently, careful analysis of the multiple copies of 16S rRNA gene alleles in *B. anthracis* and its closest relatives has revealed that the anthrax pathogen harbors a single nucleotide variant (SNV) at position 1139 in some of the alleles (derived alleles). This SNV is unique for *B. anthracis* and absent from all other members of the *B. cereus* s.l. group. The other (ancestral) allele is present in every species including *B. anthracis* and can thus serve as an internal assay control. Starting from this new understanding of the 16S rRNA gene heterogeneity in *B. anthracis* we developed a novel fluorescence in situ hybridization (FiSH) assay for detection by differential labelling of ancestral allele is present in every species including

We tested by FiSH. Preliminary

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**IP 04**

*Pseudomonas aeruginosa* in well water in the region Rabat, Morocco: Serotyping and antibiotic susceptibility

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*Pseudomonas aeruginosa* is an opportunistic pathogen which is found ubiquitously in water and soil environments. This bacterium easily adapts to its environment by developing variants that show increased resistance towards the antimicrobial treatment. In 2013 the Center for Disease Control (CDC) in the US identified multidrug resistant *P. aeruginosa* as a serious threat, followed by a classification for carbapenem resistant *P. aeruginosa* as a critical pathogen with highest priority for research and development by the WHO in 2017. The results of our study on a large number of water samples from the wells in region Rabat, confirm the existence of frequent *P. aeruginosa* colonization (57% of examining samples). Among the 321 strains of *P. aeruginos* which benefits from a serotyping, the six most frequent serotypes are O1 (29.9%), O4 (15.0%), O5 (11.5%), O6 (7.2%), O2 (5.9%) and O16 (5%). The medium antibiotic susceptibility was: ticarcillin (61%), ticarcillin-clavulanic acid (67%), piperacillin (78%), piperacillin-tazobactam (82%), aztreonam (63%), imipenem (88%), Cefotaxime (76%), ceftazidime (76%), cefepime (68%), cefpirome (47%), gentamicin (71%), amikacin (70%), netilmicin (74%), tobramycin (76%); ciprofloxacin (66%) finally colistin (79%). Our percentage of cefepime and ticarcillin resistant strains respectively 49% and 53% is high. Interest epidemiological of serogrouping by major agglutination antigens O is now well known. In addition, our study confirmed the particularity of resistance profiles of serotype O12, which have to be controlled very carefully.

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**IP 05**

Early detection of emerging viral diseases in Tunisia: The implementation of laboratory techniques for the diagnostics of highly pathogenic viruses

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Emerging zoonotic viral diseases have been more frequently detected in recent years all over the world. Climate change, globalization and human population growth may represent the most important factors for the expansion of zoonotic diseases, changing the distribution of animal and arthropod vectors and increasing the chance of virus transmission to humans. Evidence of the presence of several potential vectors of high risk emerging arboviruses including Rift Valley fever virus (RVFV) and Crimean Congo haemorrhagic fever virus (CCHFV), as well as serological evidence of these viruses in humans and animals has been identified in Tunisia and surrounding North African countries. Therefore the risk of these emerging viral diseases occurring in Tunisia is considered to be high. Our goal is to develop a system at the Institute Pasteur Tunis (IPT) for the early detection and diagnosis of zoonotic viral pathogens in animal vectors and humans in Tunisia. A multidisciplinary team was therefore formed, consisting of personnel from the entomology, clinical virology and veterinary microbiology laboratories of IPT as part of a One Health approach. The focus of this multidisciplinary team is the implementation of effective viral diagnostic tools in close collaboration with the Robert Koch Institute (RKI) as part of the German Biosecurity Programme.

In the framework of the German Biosecurity Programme a glovebox was installed in IPT, and the IPT team participated in a practical workshop on the safe inactivation of highly pathogenic viruses. The use of the glovebox to inactivate samples should ensure the safety of staff and the environment during the detection of a range of highly pathogenic viruses at the IPT. CCHFV, RVFV, dengue virus and Ebola virus were identified as potential risks in Tunisia, and the implementation of qRT-PCR assays at the IPT for the detection of these viruses was supported by the RKI team, enabling their molecular detection. A DENV EQA was carried out to validate the efficiency of diagnostic tools in IPT laboratories, and further EQAs are planned as part of the collaboration. In order to assess the capacity of the IPT to safely and securely detect highly pathogenic viruses, a risk assessment was also carried out using BIORAM software.

Our overall goal is the effective surveillance of emerging viral diseases in Tunisia, which requires not only precise laboratory techniques but also the involvement of national networks, in a One Health approach.

**IP 06**

**Hospital emergency water supply – A hygienic-medical perspective**

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In recent years, awareness of the importance of civil protection measures has increased. In Germany, the concept of civil defense (Konzeption Zivile Verteidigung) was updated in 2016. It covers four main aspects: maintenance of governmental authority, civil protection, support of the armed forces and emergency supply. The primary goal of emergency supply is to protect the basic needs of civilians, which are drinking water, nutrition and medical care. Hospitals are key players in medical care. Therefore, they need sufficient personnel, electricity, medical consumer goods, and water.

We analyzed concepts for Hospital Emergency Water Supply (HEWS) planning. A selective literature review was performed, through which we gathered legal and political frameworks and connected them to the medical and hygienic demands of hospital water quality.

We found that existing concepts of emergency water supplies do not fit the needs of hospitals. This is because hospitals have a high water demand (up to 150 liters per patient and day) and special requirements with regard to water quality, especially in critical care patients.

Neither a supply by emergency wells nor mobile supply by water trucks nor evacuation fits the hospital’s needs with regard to water quality and quantity. Therefore, there is an urgent need for concepts that fit these special needs. These concepts have to cover all aspects of the water supply, have to take account of local conditions and must have a satisfactory cost-benefit ratio.

In conclusion, hospitals have to develop their own concepts for HEWS. The hospitals’ medical specialists in hygiene should therefore be involved as coordinators. Close cooperation between hospitals, water companies, public administration and local civil protection forces is necessary.
**IP 07**

Patient registry to evaluate safety and clinical outcomes of BAT® treatment for confirmed or suspected botulism in the United States

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**Background:** BAT® [Botulism Antitoxin HEP-TAVALENT (A,B,C,D,E,F,G)-(Equine)] anti-toxin is indicated for the treatment of documented or symptomatic botulism in adult and pediatric patients. Effectiveness of BAT anti-toxin is based on efficacy studies conducted in animal models. A 3-year observational patient registry was conducted enrolling patients treated with BAT product provided by the Centers for Disease Control and Prevention (CDC).

**Materials/methods:** A non-interventional, retrospective, phase-4 registry was designed to evaluate safety and capture clinical outcomes of BAT-treated patients. Emergent BioSolutions contacted treating healthcare providers to initiate safety surveillance and introduce the registry. Data was collected voluntarily from healthcare providers through passive data collection with active follow-up of safety data.

**Results:** This analysis includes 162 patients with a mean age of 49 years (32 days-92 years), predominantly male (61%). Nine (5.6%) patients were pediatric (32 days-14 years). AE/SAE (>3%) assessed as BAT anti-toxin-related were pyrexia (6.2%) and tachycardia (4.3%). Additional BAT anti-toxin-related AE/SAEs of special interest included brady cardia (2.5%), hypersensitivity (0.6%), serum sickness-like reaction (0.6%), anaphylactic reaction (0.6%), and dermatitis allergic (0.6%). Botulism was the final diagnosis of 69.8% (113/162) of registry patients. Duration of hospitalization was utilized as a clinically meaningful endpoint with stratification of early (within 2 days) and late BAT anti-toxin treatment. Patients treated early after botulism symptom onset were hospitalized for less time (5 days) than patients with late treatment (16 days). The same trend was observed for ICU duration; patients treated early after symptom onset were in the ICU for less time (4 days) than patients with late treatment (12 days).

**Conclusions:** Safety and outcomes were as anticipated with no new safety signals identified. Clinical outcomes are consistent with data collected pre-licensure under a CDC Expanded Access Program. This project has been funded in whole or in part with Federal funds from the US Department of Health and Human Services, Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority under Contract No.HHSO100200600017C. The content of this publication does not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

**IP 08**

MALDI-UP - the MALDI-TOF MS User Platform - Exchange of spectra to support diagnostics

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MALDI-TOF mass spectrometry (MALDI-TOF MS) is spreading rapidly in many areas of application within food control, veterinary medical diagnostics, and clinical microbiology. An unknown microorganism can be identified by comparing its mass spectrum to that in a reference spectra database. Thus, this database is the key for identification. The systems employed by most users normally offer comprehensive databases supplied by the manufacturer, but also allow to generate own spectral database entries. These entries can readily be transferred within the same device platform, making it possible to quickly fill up current diagnostic gaps. According to our experience, this is especially useful for microorganisms from clinical veterinary microbiology. Besides that, there are few databases available for applications beyond microbiology, such as the species identification of wild mushrooms, fish, insects or meat via MALDI-TOF MS, although a wide circle of users is interested in them.

In order to provide information regarding such quality-assured in-house database entries from users for users, we have set up an open catalog available at “MALDI-TOF-MS-user-platform.ua-bw.de” [1]. This non-commercial catalog contains spectra-specific information regarding species names, isolate numbers and specimens as well as details on the validity of the isolate designation and technical details of the entries (instrument, cultivation, preparation etc.). Furthermore, the platform contains also contact information about the creator of respective spectral entries, but it does not intend to provide these for free download [2].

Currently, users have entered a total of over 1,000 reference spectra entries, from which the microbiology part forms the main focus. The second area of the list includes over 2,000 individual validation spectra. These can be used for species-specific and comprehensive quality-assurance of the individual onlinedatabase [3].
The MALDI-UP list is open to further users for the purpose of mutual exchange of information on valuable MALDI TOF mass spectra on a cost-free basis.

References:

IP 09
Survival of Leptospira spp. in soil and water: A literature review

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Background: The survival time of Leptospira spp. in surface or soil water is a major factor influencing the exposure and infection risk for humans and animals. The viability of the bacteria is affected by various environmental conditions such as temperature, pH value and humidity. Several studies showed the ability of the bacteria to survive in fresh water and soil under different conditions. The aim of this review is to extract and compare previously published data on the tenacity of Leptospira spp. in soil or water.

Methodology/Principal Findings: A comprehensive literature search was performed using 5 databases (PubMed, Scopus, ScienceDirect, Google Scholar, Web of science core collection). The research was comprised with the terms “leptospira AND survival AND time”, “leptospira AND tenacity”, “leptospira AND viability”, “leptospira AND survival AND water”, “leptospira AND survival AND soil”, “leptospira AND tenacity”, “leptospira AND survival AND environment” or “leptospira AND growth conditions”.

Results: The search generated a total of 29 publications covering the period from 1918-2017. After excluding 10 studies with incomplete, irrelevant or low quality data 19 reports met the inclusion criteria. The observed survival times of leptospires were very variable. Leptospires remained viable for a very short time (<1 hour) up to several months depending on the surrounding conditions. It was shown that several factors like a neutral or alkaline pH, high humidity and no exposure to UV-light were positively associated with the survival rate. Temperature, the accompanying bacterial flora and the salinity also had an influence.

Conclusions: It is very difficult to compare the findings of different studies to each other. The studies do not only vary in their design but also the different Leptospira strains seem to adapt differently to environmental conditions. Future work should study Leptospira spp. under the same environmental conditions. This would be helpful to estimate the survival time of leptospires in the environment which determines the risk of human exposure.

IP 10
WITHDRAWN

IP 11
Q-blot: a highly sensitive immunoblot for detection of Coxiella infections

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Q fever is a world-wide occurring air-borne zoonosis caused by the obligate intracellular bacterium Coxiella burnetii. In the Western world Q fever outbreaks occur regularly but are in general small. A major outbreak affecting 50,00-100,000 people occurred between 2007 and 2011 in the Netherlands, with > 4000 disease cases. Q fever is very common around the Mediterranean, in Africa and in Australia. In the USA interest in Q fever is related to the deployment of military personnel in the Middle East.

Q fever is usually diagnosed using serology. Antibodies are distinguished based on the recognition of two different antigen subsets. Phase 1 antigens recognize specific lipopolysaccharides that are present on the outer membrane. Phase 2 antigens represent the dominant antigens present in a Coxiella strain that due to a genetic defect dose not make phase 1 LPS. The 2 antigen sets combined with the distinction between IgM and IgG distinction are the basis for evaluation of the disease stage in patients with Q fever. The Immune Fluorescence Assay (IFA, Focus Diagnostics) turned out to be most sensitive and is still used routinely.

In 2014 Innatoss participated in a field study (Morrow et al., 2015) in which > 1500 inhabitants of Herpen, the village in which the outbreak in the Netherlands was detected, were tested using IFA as well as a Q-detect™, Innatoss’ Q fever Interferon-gamma release assay.

In the Herpen study the suspicion arose that even the IFA is not sufficiently sensitive. For this reason Innatoss developed a Coxiella immunoblot for hu-
man use. Q-blot was optimized to the level that could answer the question at hand: do subjects with a positive Q-detect have antibodies that can be detected in the Q-blot, even though they are negative in IFA.

To date results show that

- Q-blot is more sensitive than IFA
- Q-blot covers a broad set of antigens
- Signals in Q-blot correlate with antibody levels in IFA
- Q blot is easier to handle and feasible for any diagnostic lab

To convert Q-blot into a diagnostic test, development of a technically stable product is ongoing as well as in depth clinical validation to establish the sensitivity and specificity parameters for 3 clinical case definitions: acute Q fever, Q fever related fatigue syndrome and chronic Q fever.

**IP 12**

A national toxin laboratory network to establish diagnostic capacities for detection of biological toxins within Germany

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Recent incidents in Europe and worldwide have highlighted the threat posed by several biological toxins (e.g. Ricin letters in the U.S. 2003/2013 and a recent event in Cologne in June 2018). Biological toxins have also been involved in naturally occurring transregional outbreaks (e.g. EHEC outbreak 2011). Hence, there is a need for increased vigilance and adequate preparedness against bioterrorism. Results from proficiency tests in the European project EQuATox as well as from a national crisis management exercise in Germany (LÜKEX 2013) indicated that biological toxin analysis at the national and federal state level can be further improved.

Therefore, a national toxin laboratory network was established which aims at implementing measures in the field of detection of biological toxins within Germany. This includes exchange of protocols and reagents to spread methods widely, standardisation of detection methods and measures for quality assurance.

In the BMBF-project SensTox the national toxin detection capabilities for 15 biological toxins were identified. This list of laboratories was further extended by surveying the federal health authorities resulting in a comprehensive overview of toxin detection capabilities in Germany. The identified laboratories comprise national and federal institutions as well as universities and companies. First measures in the laboratory network concentrated on the analysis of ricin toxin. Interested laboratories were trained in a validated and accredited sandwich ELISA method for specific detection of ricin toxin and protocols and reagents were made available to the network. Furthermore, a first laboratory exercise was organised to evaluate the successful implementation of this method in the respective laboratories.

The laboratory network will provide a basis to strengthen toxin analysis capacities in Germany, and thus will further support preparedness and response planning.

**IP 13**

Clinical and molecular diagnosis for biodefense against smallpox in Brazil

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Smallpox preparedness requires both rapid clinical and laboratorial diagnosis. To evaluate the performance of Brazilian physicians in diagnosing smallpox clinically, we used a confidential two-step questionnaire to survey 262 voluntary physicians from several states of Brazil with different medical specialties and an average age of 34 years-old. In the first part, participants had access only to the disease description and photographs of smallpox skin lesions. They were expected to recognize an exanthematic clinical case and indicate two diagnostic hypotheses. Smallpox was considered as a possible diagnosis by 45.8%, but only 31.3% indicated smallpox as the first choice. Chickenpox was considered as differential diagnosis by only 11.5%. In the second part of the questionnaire, we investigated their specific knowledge: 69% confirmed to have studied smallpox in medical school, however, the correct form of transmission was unknown by 52.3% and 51.9% did not know the correct infectiousness period. The patterns of skin lesions were known by 65.3%. These results highlight the need to reinforce medical training on the clinical diagnosis of smallpox.

In addition to that, rapid laboratory confirmation is also essential. In this regard, it is noteworthy that the orthopoxvirus (OPV) Cantagalo virus (CTGV) is endemic in Brazil causing a pustular zoonotic
disease. Infection of immunocompromised patients could be clinically misdiagnosed as a mild smallpox case. Therefore, we tested a Taqman duplex real-time PCR assay associating the specific detection of variola virus (VARV) A4L gene (Kondas et al, 2015) with the OPV F4L gene (Maksyutov et al, 2015) using 32 CTGV clinical samples. Assays were performed using the reporter dye FAM (VARV) and/or VIC (OPV) measured against CXR signal for normalization. The analytical LOD was established using a standard curve of a synthetic 330-bp DNA fragment serially diluted, containing target regions of 154bp (VARV A4L) and 176bp (OPV F4L). The LOD for both VARV and OPV were 50 molecules at Ct of 36 and 37, respectively. Control DNA samples of purified CTGV were OPV positive with Ct of 16.74, with no VARV cross-detection. CTGV clinical samples were isolated between 1999 and 2018 from cows and humans in different regions of Brazil. All were OPV positive without VARV cross-detection. To simulate a bioterrorism event, we positively tested powder samples spiked with the synthetic DNA fragment before and after DNA extraction.

Results:

The adapted and validated protocols resulted in a $\geq 4 \log_{10}$ reduction of spores, mycobacteria, and viruses at all selected locations within the animal room as well as the corresponding HEPA filter unit. The temperature of the surfaces as well as the soil load, respectively, had an unexpected mitigating and enhancing influence. Commercially available germ carriers might indicate false negative results. Furthermore, using the identified effective concentration of PAA, no damage of electronic devices could be observed so far.

Conclusion: PAA decontamination protocols are highly effective in rendering an area or device safe to handle. Unfortunately, each environment has to be specifically validated on relevant surfaces and at multiple locations with representative suitable surrogates for the microorganisms to be handled. Using only a small number of germ carriers or inadequate surrogates might generate misleading results.

IP 14
Development of large-scale peroxyacetic acid based aerosol decontamination protocols in practice

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Background: The adaption of airborne disinfection protocols is influenced by a variety of parameters, e.g. surface temperature, room humidity (rH), or presence of microorganisms in organic matter. Therefore, our adaption process was of upsampling nature, beginning with biosafety cabinets and ending up with complete large animal rooms.

Materials and Methods: Enveloped and non-enveloped viruses, spore forming bacteria, and mycobacteria with and without soil load were inoculated on stainless steel carriers according to quantitative carrier testing protocols (DVG, RKI guidelines). The carriers were placed at different locations within a containment animal room (245 m²). The room was aerosolized with ultradine particles (7.5 µm) of a solution containing 1.3 % peroxyacetic acid (PAA) until a high rH was reached. After an incubation time of 30 min and an aeration phase, the microorganisms were recovered and the inactivation efficacy was determined as $\log_{10}$ reduction. A similar procedure was chosen for the high efficiency particulate air (HEPA) filter system of the animal room.

Results:
The adapted and validated protocols resulted in a $\geq 4 \log_{10}$ reduction of spores, mycobacteria, and viruses at all selected locations within the animal room as well as the corresponding HEPA filter unit. The temperature of the surfaces as well as the soil load, respectively, had an unexpected mitigating and enhancing influence. Commercially available germ carriers might indicate false negative results. Furthermore, using the identified effective concentration of PAA, no damage of electronic devices could be observed so far.

Conclusion: PAA decontamination protocols are highly effective in rendering an area or device safe to handle. Unfortunately, each environment has to be specifically validated on relevant surfaces and at multiple locations with representative suitable surrogates for the microorganisms to be handled. Using only a small number of germ carriers or inadequate surrogates might generate misleading results.

IP 15
State of the art decontamination

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To inactivate microbiological contaminants inside safety cabinets, isolators and air locks the conventional wipe disinfection is not sufficient. In particular, filters but also hard-to-reach areas cannot be reliably decontaminated in this way. Therefore, relevant regulations and standards stipulate that vaporized or nebulized agents should be used for decontamination.; The most common gaseous or aerosolized agents for room decontamination are ethylene oxide, formaldehyde and hydrogen peroxide. All substances are characterized by a high biological effectiveness but differ in regard to conditions of use, exposure time and health risks. An increasingly important aspect is the fact that ethylene oxide and formaldehyde are considered to be carcinogenic. In contrast, hydrogen peroxide is classified as less harmful to human health and to the environment. Mainly due to its low toxicity as well as to its relative ease of use, hydrogen peroxide has become the method of choice in many laboratories and in industrial processes.; Despite the principal advantages of hydrogen peroxide it is still a complex issue to develop a suitable, reproducible and validated decontamination procedure even with this agent. A number of factors like temperature,
humidity, vapourisation method, aeration and de-
composition have to be taken into account to make
the process sufficiently effective, compatible with
all materials, less time consuming and safe. Most
important becomes the design of the hydrogen per-
oxide generator for fast and even distribution inside
the containment, while avoiding leakage into the
environment.

In various regulations, recommendations and stan-
dards information on implementation and the cor-
rect validation can be found. Nevertheless, many
users may not be sufficiently informed about the
possibilities and requirements for the decontami-
nation of containments used for microbiological,
biotechnological or medical work.

In this presentation we will describe requirements
and basic conditions for the decontamination of
safety cabinets, isolators and air locks. We aim
to provide an overview of the current state of im-
plementation and to give an outlook on future
developments.

Aims & Tasks of NaLaDiBA: The main goal
of the network is to improve the ability to respond
to biological threats and thus ensure a better pro-
tection of the population at risk from biological
materials like poxviruses or anthrax. Next to the
cross-linking of laboratories within Germany, the
main tasks include updating, extending and har-
monizing existing detection procedures, providing
training courses, and performing quality assessment
studies.

Achievements: In the past years, the network has
set up a webpage and a leaflet with information
about the network for the general public. In sev-
eral meetings the project status, tasks, goals, and
activities were discussed. The network conducted
four training courses, performed three ring trials,
participated in a proficiency test for Ebola virus di-
gnostic during the outbreak in West Africa, tested
the Razor EX mobile PCR analyzer, developed and
shared standard operating instructions for 13 PCRs
detecting different bacterial and viral pathogens,
and compared their performance on different PCR
platforms. Additionally, an internal control was
developed and distributed among the network mem-
bers. In cooperation with altona Diagnostics GmbH
a real-time PCR kit for the detection of *Bacillus
anthracis* was developed and validated.

Conclusion & Outlook: The network has estab-
lished and implemented a range of different PCR
assays for the detection of highly pathogenic agents
(e.g. during the Ebola virus outbreak 2014/15), has
trained the procedures in wetlabs, tested the labo-
raries’ performances in ring trials and is therefore
well prepared for the diagnostics in extraordinary
biological risk situations. Furthermore, the benefit
of the network has been proven during the initial
phase of the influenza pandemic when one of the first
German-wide real-time reverse transcriptase PCR
assays for the detection of Influenza A H1N1pdm09
was established by the network members.

As the list of pathogens causing an extraordinary
biological risk situation is versatile, the panel of de-
tectable pathogens needs to be updated constantly
and the work on virtuoso diagnostic methods will
be continued.

**IP 16**
Consolidation of NaLaDiBA (Nationale
Labornetzwerk für die Diagnostik von BT-
Agenzien) – Achievements and tasks for
the future

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Introduction: NaLaDiBA, existing since 2009,
is a network of currently 11 national laboratories
in Germany working on the detection of highly
pathogenic agents in extraordinary biological risk
situations. In the beginning of 2018, the network
was consolidated with a permanent contact person
at the Robert Koch Institute who is coordinating
the network.

The network has established and implemented a range of different PCR
assays for the detection of highly pathogenic agents
(e.g. during the Ebola virus outbreak 2014/15), has
tained the procedures in wetlabs, tested the labo-
raries’ performances in ring trials and is therefore
well prepared for the diagnostics in extraordinary
biological risk situations. Furthermore, the benefit
of the network has been proven during the initial
phase of the influenza pandemic when one of the first
German-wide real-time reverse transcriptase PCR
assays for the detection of Influenza A H1N1pdm09
was established by the network members.

As the list of pathogens causing an extraordinary
biological risk situation is versatile, the panel of de-
tectable pathogens needs to be updated constantly
and the work on virtuoso diagnostic methods will
be continued.

**IP 17**
On the efficiency of different recirculating-
type air disinfection devices

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It is most critical to ensure aseptic conditions for many facilities. So, bioaerosol removal from indoor air is an urgent task. The market offers a wide variety of flow through type air disinfectors now, which are tested for filtration efficiency according to the available standards (AHAM: Standard Test Procedure ANSI/AHAM AC-1, 2002), based on the Clear Air Delivery Rate value. But thus value does not say anything about the inactivation of microorganisms that have passed through disinfectors, which results in the reduction of the concentration of viable microorganisms at the device outlet not only due to the retention of particles in the device. We believe that a natural measure of the efficiency of aerosol disinfection by the test air disinfection devices (ADD) $E_b$ is the ratio of the concentrations of viable microorganisms at the device output $C_{b, out}$ and inlet $C_{b, in}$:

$$E_b = \frac{100}{100} \left( 1 - \frac{C_{b, out}}{C_{b, in}} \right), \text{%}.$$

Very important ADD’s efficiency characteristic is filtration efficiency $E_m$. The relationship based on the ratio of aerosol mass concentrations at the device output ($C_{m, out}$) and inlet ($C_{m, in}$) is proposed:

$$E_m = 100 \left( 1 - \frac{C_{m, out}}{C_{m, in}} \right), \text{%}.$$

One can calculate efficiency of microorganisms passing through ADD inactivation $E_i$ from formulas above:

$$E_i = 100 \left( 1 - \frac{1 - E_b}{100 \text{%}} \right) \left( 1 - \frac{C_{m, out}}{C_{m, in}} \right), \text{%}.$$ 

The $E_i$ value shows percentage of microorganisms which lost their viability during passing through ADD out of all microorganisms passed ADD. 

While the proportion of the total number of aerosol particles that passed through the device only weakly depends on indoor temperature and relative humidity (except for their extreme values), for viable microorganisms such dependencies can be very sharp. Moreover, various microorganisms differently respond to inactivating factors inside the device such as ozone, UV-radiation, the gas phase composition, etc.

Obviously, it is correct to compare the efficiencies of air disinfection by different devices only on condition of identical microorganisms aerosolized from the same material (liquid or dry) under identical microclimatic conditions. In all other cases, the comparison of the efficiencies of various ADD will be incorrect.
**IP 19**
Influence of decontamination techniques on *Brucella* spp. identification by MALDI-TOF-MS

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**Background:** MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) has gained increasing importance for rapid bacterial species identification in routine diagnostic laboratories. Since MALDI-TOF MS devices are usually located outside of biosafety level 3 laboratories, complete inactivation of highly pathogenic bacteria prior to analysis is indispensable. However, altered mass spectrometry profiles can be expected dependent on the decontamination technique applied. Therefore, we assessed their impact on bacterial species identification by MALDI-TOF MS.

**Materials and Methods:** Heat, ethanol, 2-propanol, glutaraldehyde, formaldehyde, hydrogen peroxide, peracetic acid, sodium hypochlorite or trifluoroacetic acid were applied to kill *Brucella* spp. Following decontamination, samples were prepared for MALDI-TOF MS using ethanol/formic acid extraction and analyzed with a Bruker MicroflexLT mass spectrometer. Mass spectra were classified by hierarchical cluster analysis and compared to reference spectra databases using Bruker Biotyper, the R package MALDIquant and the Mass-Up software.

**Results:** The use of sodium hypochlorite and glutaraldehyde led to insufficient spectral quality in terms of peak number and intensity. Hierarchical cluster analysis of the other spectra divided the preparations into two major clusters containing heat, ethanol, 2-propanol as well as trifluoroacetic acid on one hand and peracetic acid and hydrogen peroxide on the other hand. Formaldehyde did not match any of these clusters. The analysis of mass spectra using Bruker Daltonics’ as well as our in-house *Brucella* database identified (Bruker Biotyper score > 1.7) *Brucella* spp. only in case of heat, ethanol, 2-propanol and trifluoroacetic acid preparations.

**Conclusion:** Choosing a suitable decontamination technique is crucial for the successful identification of highly pathogenic bacteria using MALDI-TOF MS since it may significantly influence the quality and information content of mass spectrometry profiles. Therefore, the methods used to create reference databases and those applied in routine diagnostics should be identical or must be carefully validated in advance to allow successful identification.

**IP 20**
Culture-independent serotyping of *Legionella pneumophila* in water and urine samples for faster risk assessment of *Legionella*-exposure

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Increasing numbers of legionellosis cases within the last years have shown that an innovative, fast and sensitive method for the detection of *Legionella pneumophila* in water (e.g. surface water, drinking water, process water) and urine samples is still needed. Currently, cultivation of clinical and environmental samples represents the gold standard method for the detection of *Legionellae*. This method takes 10 days and only detects culturable *Legionella* spp. Subsequently, further methods have to be applied in order to type obtained *Legionella* colonies. A special focus lies on a complete serotyping of *L. pneumophila* Sg 1 - 15 as legionellosis is mostly caused by the species *L. pneumophila* (90%). During outbreak situations caused by emissions of *Legionella*-containing aerosols from condensation recooling plants to the environment, it is essential to rapidly link infected patients to the outbreak source in order to prevent further cases. For this reason, a chemiluminescence sandwich microarray immunoassay (CL-SMIA) was established in order to detect and serotype *L. pneumophila* directly in liquid samples on the microarray analysis platform MCR-R. A rapid and multiplexed detection method for serotyping of all serogroups and subgroups of *L. pneumophila* within only 34 min is possible with a panel of 19 sensitive and selective monoclonal antibodies. The CL-SMIA can be applied in various settings such as in clinical diagnostics and environmental hygiene monitoring of the health authorities to analyse water samples as well as patients’ urine samples. Here, a direct and rapid comparison of patient’s urine samples with environmental water samples of putative outbreak sources can be done. This allows to rapidly exclude of many environmental samples and further laborious analytical
methods and to focus on a smaller number of samples during outbreak investigations.

**IP 21**

Microarray-based rapid verification, quantification and risk assessment of *Legionella* by heterogeneous amplification and viable/non-viable differentiation

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Based on the 42nd Federal Emission Control Act (42. BImSchV) condensation recooling plants, cooling towers and wet separators have to be registered since 2017. This also implies a regular monitoring of the number of Legionella in the process water. Today, cultivation is still the gold standard for the detection of Legionella, though it takes 10 days and only a general detection of *Legionella* spp. is possible. As legionellosis is often caused by the highly pathogenic species *Legionella pneumophila* (90%), it is important to quantify *Legionella* spp. and *L. pneumophila* culture-independent and rapid. Additionally, a viable/non-viable differentiation is necessary to monitor biocide effects. For this reason, a chemiluminescence DNA microarray on the automated flow-based analysis platform MCR 3 was established for the isothermal heterogeneous asymmetric recombinase polymerase amplification (haRPA). For quantification and differentiation of *Legionella* spp. and *L. pneumophila*, the genomic sequences of 16S rRNA and mip gene were used, respectively. To differentiate between viable and non-viable Legionella, the sample is pretreated with the DNA-intercalating dye propidium monoazide (PMA), which intercalates exclusively into the DNA of non-viable Legionella with permeable cell membranes. By subsequent haRPA only viable Legionella are detected. Calibration curves for the haRPA were obtained with specific RPA primers, reaching detection limits of 87 genomic units (GU) µL⁻¹ for *Legionella* spp. and 26 GU µL⁻¹ for *L. pneumophila*. With the viability haRPA, predefined proportions of viable Legionella could be measured in a range from 10¹ - 10⁹ GU µL⁻¹ with recovery rates of 81 to 133%. A combination of both methods allows the quantification of the sum parameter of viable and non-viable Legionella and the determination of the proportion of viable Legionella in the sample on one microarray with two flow cells in less than one hour. For increase of sensitivity, the method was combined with the preconcentration method monolithic adsorption filtration (MAF) to make it applicable to regular monitoring of condensation recooling plants. In previous experiments, process water was measured within a few hours and the effect of biocides was demonstrated.

**IP 22**

Production and characterization of a *Bacillus anthracis* secretome with suitable characteristics as antigen in serological tests

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Introduction: Bacillus anthracis, the causative agent of anthrax, produces a complex secretome which contains the well known toxin proteins lethal factor (LF), protective antigen (PA) and edema factor (EF) and many other proteins, such as proteases and degradative enzymes, which are able to modulate the composition of B. anthracis secretome during the infection.

Materials and Methods: In this study, we cultured the vaccine strain *B. anthracis* Sterne 34F2 in media containing EDTA and assessed the best conditions to inhibit the activity of zinc-dependent metalloproteases and to obtain a secretome containing high concentration of not degraded PA (PA83), as evaluated by the SDS-PAGE analysis. Then, we used this secretome, named PAS, as antigen in a Complement Fixation Test (PAS-based CFT), to monitor the production of antibodies to PA83 in serum samples of rabbits vaccinated with Sterne 34F2 and then infected with a B. anthracis virulent strain, as prescribed to evaluate the potency of the vaccine. The PAS-based CFT results were compared with those obtained by using a commercial ELISA kit that employs purified toxin PA83 as coating antigen.

Results: The two serological tests gave similar results in terms of specificity and sensitivity, as the kinetic of antibodies production was very similar. Based on our results, the Sterne 34F2 vaccine induced an antibody response to PA83 whose titer was not inferior to 1: 8 in PAS-based CFT and 42 kU/ml in PA83-based ELISA, respectively, in all vaccinated rabbits surviving to the anthrax infection.

Discussion: Since CFT offers many benefits compared to ELISA, our opinion is that the PAS-based CFT could be successfully employed in humans and in animals for anthrax infections retrospective assessments, differential diagnosis considerations or
post vaccination monitoring. In particular, we suggest the use of our method to measure the efficacy of veterinary anthrax vaccines in replacement of the experimental infection with B. anthracis virulent strains (challenge test), as actually prescribed, which exposes laboratory technicians to many risks and causes considerable sufferings to animals. Finally, experiments are in progress in our laboratory to evaluate the immunogenic characteristics of the purified PAS, to verify its possible use as candidate vaccine/adjuvant against anthrax.

IP 23
TickKitqPCR® – Real-time multiplex PCR kit for detection and identification of viral (CCHFV and TBEV) and bacterial (Francisella tularensis and Borrelia burgdorferi) agents transmitted by ticks
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In 2008 ECDC emphasized the importance of vector-borne diseases, implying that it is necessary to strengthen the preparedness and response for such diseases. The devastating military impact of vector-borne diseases has been well documented in the scientific literature. Arthropod vectors are particularly dangerous as biological warfare agents as they are easy to sneak across borders, reproduce quickly, and spread disease in the spreading area.

Over and above that, the climate disorder is a factor that is going to impact deeper on human health as the life cycle and the area of distribution of vectors will be modified, and with them the epidemiology of vector borne diseases. This will render these biological agents even more suitable for bioterrorist attacks. Based on the rate of transmissibility, infected dose, economic impact and the available vaccines the Tick-Borne Encephalitis and Crimean-Congo Haemorrhagic viruses were classified in C Category, while Francisella tularensis was classified as an A Category agent. Those categories contain the biological agents potentially used as as bioweapons in a bio terrorist attack.

TickKitqPCR® is an in vitro PCR nucleic acid amplification test for qualitative and quantitative detection of genomes of Borrelia burgdorferi s.l., Francisella tularensis, tick-borne encephalitis virus (TBEv), and Crimean-Congo haemorrhagic fever virus (CCHFv), in biological materials (ticks, blood, cerebrospinal fluid, and animal tissue), by using real-time hybridization-fluorescence detection. TickKitqPCR® will contribute significantly to the national societal objectives of security, and health maintenance, through the generation of a rapid pathogen detection tool.

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IP 24
WITHDRAWN

IP 25
Comparison of commercial DNA extraction kits for extraction of bacterial genomic DNA
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In the past decades the use of nucleic-acid-amplification techniques for the detection of infectious agents in clinical and environmental specimen has undergone major improvements in terms of speed, throughpup, and associated costs. In this regard, the extraction of nucleic acids plays a vital role for many downstream applications in molecular biology, such as polymerase chain reaction (PCR) amplification, blotting analysis and genomic-library construction. While much research projects have focused on novel PCR applications, less information is available addressing samples processing for optimal DNA recovery prior to amplification.

For a reliable detection of microbial DNA, robust extraction methods are required. Usually, commercially available DNA extraction kits are preferred, as they provide superior reproducibility, quality control, and the potential for automatization. These kits rely on different principles for DNA purification, including solid-phase extraction techniques (e.g. anion-exchange methods, silica-membrane
technology and magnetic-particle technology) and solution-based (salting out) protocols, that are based on precipitation of DNA. Furthermore, the presence of contaminants like proteins or other cell components can cause further challenges to nucleic acids extraction, quantification, and amplification and may interfere with enzymes used in downstream applications.

The goal of this study was to compare the effectiveness of 19 commercial DNA extraction kits to extract pure, high-quality bacterial DNA from the Gram-negative bacterium Francisella tularensis and from the Gram-positive bacterium Bacillus anthracis. Extractions were performed according to the manufacturers’ protocols, and DNA was eluted or redissolved, respectively, in nuclease free water. The quality and quantity of the extracted DNA was subsequently assessed by spectrophotometric measurements (NanoDrop), Qubit measurements and real-time PCR amplifications. Two real-time PCR analyses, one targeting the dhp61 gene of B. anthracis and the other the 16S rRNA gene in F. tularensis, were conducted in order to evaluate the presence of amplifiable DNA and the removal of PCR inhibitors in the extracts. Although all methods produced suitable DNA for challenging downstream analyses, the Epicentre MasterPure Complete DNA and RNA Purification Kit gave the lowest Ct values for both, F. tularensis and B. anthracis-PCR, and the highest DNA yield within comparable short hands on time.

**IP 26**
An improved DNA extraction protocol from Bacillus anthracis spores using bead-based mechanical and chemical lysis

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Bacillus anthracis, a Gram-positive bacterium and the causative agent of anthrax, has been adapted for use in biological warfare programs, bioterrorism and biocrimes in the past. It shows a high tenacity, which is attributed to the formation of endospores. The complex, multilayer structure of Bacillus spores features an outer cortex and a coat that are resistant to chemical and physical treatments. Current identification methods for Bacillus spores include conventional culturing and biochemical or serological analysis after germination of the spores. This is done at the cost of analysis time because results take at least two days for completion.

Various methods have been used to optimize the accessibility of Bacillus spore DNA to increase the sensitivity of diagnostic DNA-based methods like polymerase chain reaction (PCR). This includes

- A) heat treatment,
- B) chemical agents, such as detergents and chaotropic salts,
- C) mechanical disruption, e.g. sonication
- D) enzymatic treatment.

However, most spore lysis techniques for Bacillus have not been evaluated regarding their quantitative efficiencies or just detected DNA externally attached to the spores.

In this study, an easy-handling and time-saving bead-based mechanical lysis protocol has been developed and evaluated in order to extract amplifiable, high-quality DNA from within B. anthracis spores that were previously devoid from externally attached DNA.

Efficiencies of spore lysis were verified by transmission electron microscopy and treatment of spores with the DNA-binding, fluorescent dye propidium monoazide (PMA). Finally, the yield of genomic spore DNA in lysates was quantified by real-time and digital droplet PCR. Efficient DNA extraction was dependent on

- A) the composition of the lysis matrix, including size, material and relative content of microbeads,
- B) instrumental parameters, such as duration and frequency of agitation and
- C) the method used for subsequent DNA purification.

The final standardized lysis and DNA extraction protocol allows quantitative detection of low levels (<100 CFU/ml) of B. anthracis spores and is suitable for direct quantification of Bacillus spores even under resource-limited field conditions, where culturing is not an option.

**IP 27**
Simultaneous detection and definitive identification of pathogens using sequencing-by-hybridization

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Many molecular diagnostic methods have been developed for detecting RNA viruses. While these methods are highly sensitive and specific, they are designed to detect a single or a limited number of sequence targets within the genome, and do not distinguish variant sequences among amplified products. Here, we describe an assay, based on sequencing-by-hybridization (SBH), for simultaneous detection and definitive identification of multiple species and strains of RNA viruses. SBH is a well-known method for reading unknown DNA,
and therefore, can be used to detect and identify pathogens without prior knowledge of their identities. In its current format, the assay can identify up to 199 virus strains or isolates representing 35 species in nine genera and five families (Arenaviridae, Bunyaviridae, Filoviridae, Flaviviridae, and Togaviridae). This approach was evaluated with blinded samples that contained different species of old- and new-world arenaviruses. Each sample was tested individually against 199 viral strains or isolates on a single microarray, and the results indicated that it was possible to correctly identify the viral species or strain in the blinded sample with high accuracy. This approach has the potential not only to accurately identify known viral pathogens, but also to detect variant sequences of emerging or genetically engineered viruses.

**IP 28**

Comparison of sensitivity of commercial available lateral-flow immunoassays for *Yersinia pestis*, *Francisella tularensis* and *Bacillus anthracis* using live BSL-3 strains

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Rapid detection of biological agents in the matter of biodefense is critical on the operational, tactical and strategic level as well as for medical countermeasures. In this context *Yersinia pestis*, *Francisella tularensis* and *Bacillus anthracis* still belong to the most relevant bacterial agents in biological warfare or bioterrorism. For their detection immunochromatographic lateral flow assays are used by many response forces and several companies offer such rapid tests. Hence, it is important to know the limitation of the tests. There are few previous publications on the sensitivity of different rapid tests. However, most of these studies used inactivated bacteria, which could have impact on the test performance. In this study our aim was to determine and compare the limit of detection of several lateral flow immunoassays using live BSL-3 strains. We evaluated tests from 4 different companies (New Horizons, Tetracore, Advnt, Miprolab) for the following bacteria: *Y. pestis*, *F. tularensis* and *B. anthracis*. Furthermore, we wanted to evaluate the influence of different matrices on the performance of the tests. Liquid cultures were grown in respective media and culturing conditions. Quantification of the vegetative bacteria in the McFarland standard was done by plating and counting colony forming units. *B. anthracis* spores were purified and quantified microscopically and by counting colony forming units, respectively. Rapid tests were done with a dilution series up to 1:1000. Finally, the results were compared to quantitative real time PCR.

**IP 29**

Performance of dried real-time PCR reagents in biothreat detection

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**Introduction**: Rapid detection and accurate identification of infectious agents are essential in the response to disease outbreaks and biothreats. In recent years, reliable and fast methods have evolved enormously. However, technical limitations may restrict the use of these methods outside of sophisticated laboratories. Nucleic acid amplification requires specific chemistry and often a cold chain is needed. Stabilized reagents and assay kits stored at room temperature would enhance field utility of such detection methods.

**Materials and Methods**: Vacuum- and lyophilization drying techniques were used to make real-time PCR reagents more stable at room temperature (RT) and +4 °C. Performance of dried real-time PCR assays for influenza A virus detection were compared. Reagents containing fast 1-step RT master mix, target specific oligonucleotides and sugars (trehalose and dextran) were vacuum-dried on real-time PCR plates, and lyophilized PCR reagents, containing 1-step RT master mix, RNase inhibitor and target specific oligonucleotides were freeze-dried inside small glass vials. Dried PCR products were stored at RT, +4 °C and -20 °C and sensitivity and sustainability of the assays were tested during a 12 week period. Tenfold diluted Influenza A RNA was used to investigate the limit of detection with various time points. All real-time PCR runs were performed using QuantStudio®5 Real-Time PCR System (Life Technologies).

**Results**: Both drying techniques exhibited similar assay sensitivity. Vacuum dried reagents were stable at -20 °C and +4 °C for 12 weeks and at RT for four weeks. Lyophilized reagents were stable in all tested storage temperatures for 12 weeks. Real-time PCR runs with vacuum-dried and lyophilized assays could be completed within 45 min and 2h, respectively.

**Discussion**: Dried reagents proved to be easy to use since the assays are ready to use in a plate or a glass vial. This minimized pipetting errors and also reduced the need for highly trained person-
nel. The results demonstrate potential feasibility of vacuum-dried and lyophilized reagents to be used in field for rapid detection of pathogens. Furthermore, cold chain is no longer necessary for real-time PCR reagents in field. Field capable reagents can provide advanced technical support in remote locations where stationary laboratories are unavailable. Development of dried real-time PCR assays for other pathogens is in progress.

**IP 30**

**Distribution of ricin in whole blood – implications for detection methods**

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Ricin, the toxic ingredient of the seeds of _Ricinus communis_, is one of the most potent biological toxins known. Due to the ease of availability of the toxin bearing castor beans it has gained attention as a biological warfare agent and nowadays especially as a potential bioterroristic agent. After intoxication clinical symptoms are mainly non specific, so the confirmation of contact to ricin by laboratory analysis of clinical specimen is of major importance. While the plant alkaloid Ricinine can be detected in blood and urine for days after the intoxication it might be absent when highly purified Ricin was used. So, the direct detection of Ricin in clinical specimen is needed.

We invented the detection of Ricin by magnetic enrichment followed by enzyme linked detection, a so called “On-Bead ELISA”. Since sensitivity of the assay in plasma derived from Ricin-spiked blood samples was worse compared to spiked PBS, we investigated the binding of Ricin to leucocytes via flow cytometry and found that Ricin with its lectin moiety binds to all leucocytes, especially to macrophages. Its binding even to erythrocytes has been demonstrated earlier. Therefore, pre-analytical procedures for the preparation of blood samples before they are analysed in the “On-Bead ELISA” were tested for their ability to positively affect the sensitivity of the detection of Ricin in this important clinical matrix.

**IP 31**

**Screening test for determination of Francisella tularensis virulence in vitro**

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The causative agent of tularemia - _Francisella tularensis_ - is characterized by high pathogenicity for a wide range of hosts including humans. Moreover, the literature does not describe the cases of isolation of avirulent strains from nature or from animals. But in the laboratory experiments the investigators working with mutants are faced with the phenomenon of bacterial virulence loss. For example, it was shown that the acquisition of antibiotic resistance is accompanied by a decrease of bacterial virulence for sensitive hosts. Quite often, the attenuation process of pathogenic agents leads to changes in lipopolysaccharide (LPS). However, the population of the mutant strain may be heterogeneous in virulence. Meanwhile population-based analysis of altered subcultures with the help of biological models is expensive, time-consuming and inhumane. This problem determines the necessity of the simple methods development for the evaluation of the tularemia agent virulence without the use of laboratory animals (in vitro). The development of such a method was the purpose of this study. Previously, it was reported that we obtained the isogenic avirulent mutants (DCL ≥ 10⁶ CFU/mice) from highly virulent parent strains of the three main subspecies which have typical S–form LPS. A more detailed study of the mutants revealed that all of them had different damages in the LPS structure. As it is known, LPS is a specific immunodominant antigen and it is widely used for _F. tularensis_ identification and diagnostics, including immunochromatographic tests (IC-test). We have studied the possibility of the application of IC-tests designed to detect _F. tularensis_ LPS for differentiation of virulent and avirulent strains. Six virulent strains (A-Cole, AE-261 _subsp. tularensis_; 543, 240 _subsp. mediiasiatica_; 503, 250 _subsp. holarctica_) and their eight isogenic avirulent mutants were investigated in the present work. It was shown that all the parent virulent strains gave two bands (control and LPS-antigen specific), while all avirulent mutants gave only one band (control) and LPS-specific band was absent. Thus, the IC-test-based method allows to evaluate the _F. tularensis_ virulence in vitro and minimizes the use of laboratory animals.
Microbiological characteristics of *Bacillus anthracis* in Kazakhstan and perspectives of genotyping

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*Bacillus anthracis* is ranked high on the list of select agents for bioterrorism because of its virulence, the stability of its spores and the ability of aerosolized spores to infect humans. As a zoonotic disease, anthrax infection in humans typically occurs after butchering of infected animals. In Kazakhstan anthrax reemerged in 2016 at several regions: Almaty, Karaganda, Pavlodar and East Kazakhstan, within a short period, in places where the disease has not been registered for decades. From 19 infected people 3 casualties were reported. In Kazakhstan there are (from 1948 to 2016) 1786 stationary anthrax settlement points, 2616 anthrax foci which resulted in 25,313 livestock losses and 1907 registered human cases of the disease. From anthrax outbreaks in 2016, a total of 42 cultures were isolated. Samples were taken, where anthrax infection was confirmed, from people, animal meat, swabs, soil, and manure. Positive environmental samples were between 0.2 and 1.7%, positive meat was 30.1% and hides 4%; Atypical strains of *B. anthracis* from meat or soil from Almaty and East Kazakhstan regions were identified. In some strains spore formation occurred already after 3 hours in liquid broth and after 6-24 hours on solid media. In contrast, spore-forming from meat sample isolates only occurred after 8-14 days. The reason for the observed delay in spore-formation is not yet clear. In situations of crisis, it is crucial not to be limited to standard culture methods for pathogen identification. It is important to apply molecular-biological methods for diagnosis, to determine isolates’ genotypes and to compute phylogenies that will allow for predictions of the pathogen’s regional origin or importation events. Until 2004, there was no genetic characterization of Kazakh *B. anthracis* strains. Analysis of strains from the collection of the M. Aikimbayev’s Kazakh Scientific Center for Quarantine and Zoonotic Disease within the project the Civilian Research Development Foundation for (CRDF US) KZ-1950-AL-03 by using multilocus variable number tandem repeats (MLVA 8) revealed 5 clusters and 12 genotypes circulating in Kazakhstan. In a next step we will study anthrax soil foci to define (genomic) genotypes of *B. anthracis* with the objective to elucidate the zoonotic situation in various regions of Kazakhstan.

**JP 02**

Multipoint analysis of anthrax strains isolated in 2016 in Kazakhstan

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**Introduction:** Annually human cases of Anthrax are registered in Kazakhstan. In 2016 the Anthrax was registered in the East Kazakhstan oblast, Almaty oblast, Pavlodar oblast and Karagandy oblast of Kazakhstan. The important step of epidemiological monitoring is genotyping of Anthrax strains for further pathogen identification and determination of geographic origin.

**Aim of study:** The aim of study is to investigate the genotypes and genetic peculiarities of anthrax strains isolated in Kazakhstan with MLBA-25 and provide the map of identified Anthrax strains distribution for further investigation the possible link between *B. anthracis* and its geographical origin.

**Materials and methods:** Epidemiological, epizootiological, microbiological, genetic methods of investigation were used during the study.

**Results:** The analyzed strains were clustered into 6 genotypes and three clades during the MLVA typing for 25 VNTR loci and phylogenetic analyses. The strains from the East Kazakhstan and Almaty oblast are located within the first clade. These strains are grouped next to the European (France, Germany), Asian (Tajikistan, Pakistan, Korea) and African (Namibia) strains. This group is presented by strains belonged to A line of A.Br 008/009, A.Br.Volum and A.Br.WNA by canSNP Sublines. The strains from Karagandy oblast are located within the second clade, these strains are clustered next to the strains from Japan. The strains from Pavlodar oblast and the strains from Karagandy oblast belonged to B group and are located within the third clade. In the previous
studies B group did not register in Kazakhstan. These strains are grouped with the Strains from France and Germany and belonged to B/Br.CNEVA and B.Br.001/002 according to CanSNP.

**Conclusion:** Molecular-genetic analysis of *B. anthracis* increases the capacity of epidemiologists to monitor the source and routes of the infection. The modern molecular-genetic methods of investigation will improve the surveillance system of highly dangerous pathogens in Kazakhstan.

**JP 03**

**Phylogenetic relationships and genetic diversity within the most comprehensive collection of Clostridium botulinum group I and the close related species Clostridium sporogenes**

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**Background and objectives:** Accurate surveillance of bioforensic pathogenic bacteria demands a clear taxonomical assignment. Historically, the name 'botulinum' was assigned to *Clostridium* strains producing the neurotoxin, independently from the phylogenetic background. The major aim of our study was to analyse a comprehensive collection of *C. botulinum* group I and *C. sporogenes* in order to identify the correct taxonomic assignment of each isolate and to provide a more robust picture of the overall population structure of these two closely related species as well as the neurotoxin subtype distribution.

**Material and methods:** 84 newly sequenced genomes and 136 publicly available whole-genome sequences of *C. botulinum* and *C. sporogenes* were used. Isolates showing an average nucleotide identity higher than 95% were regarded as the same species. Phylogenetic analysis was performed using Roary and RAxML software, while neurotoxin subtype distribution was assessed by means of an in silico Megablast-based approach.

**Results:** Three major clusters and three different species, namely *C. botulinum* group I, *C. sporogenes sensu stricto* and *C. sporogenes sensu lato*, were observed ad misclassified isolates were identified. Both, *C. botulinum* and *C. sporogenes* species include members producing or not producing the neurotoxin. For *C. botulinum* group I, a correlation between neurotoxin subtype and phylogenetic background was observed. Among *C. sporogenes* isolates only neurotoxins type B were detected.

**Conclusion:** This work contributes to a better understanding of *C. botulinum* group I and *C. sporogenes* population structure in terms of genetic diversity and neurotoxin distribution. Furthermore, our data suggest that *C. sporogenes* taxonomically represents two different species.

**JP 04**

**Mapping the spread of Bacillus anthracis across the Southern Caucasus using novel genomic single nucleotide polymorphisms**

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The zoonotic disease anthrax caused by *Bacillus anthracis* is common in the southern Caucasus area. From a population genetics view the major canonical single nucleotide polymorphism (canSNP) groups A.Br.Aust94 and A.Br.008/009 dominate in Turkey and the republic of Georgia. Thus far, isolates of *B. anthracis* from Turkey have been genotyped predominantly by multi locus variable number of tandem repeat analysis (MLVA) or canSNP typing. While whole genome sequencing is the future gold standard, it is currently costly on a broader scale. For that reason we were interested in identifying novel SNPs which could facilitate efforts to further distinguish closely related isolates using low cost assay platforms. In order to achieve this goal, we sequenced the genomes of seven strains of *B. anthracis* collected from the Kars province of Eastern Anatolia in Turkey and discovered new SNPs which allowed us to assign these and other geographically related strains to three novel branches of the major A-branch canSNP-group (A.Br.) Aust94. We named these new branches Kafkas-Geo 1-3 comprising isolates from the Kars region and the neighboring republic of Georgia suggesting a common ancestry. The novel SNPs identified in this study connect the population genetics of *B. anthracis* in the South Caucasus and Turkey. We expect this knowledge to assist efforts to map the transboundary spread of the pathogen in this region.

**JP 05**

**Comparative phylogenetic analysis of Francisella tularensis subsp. holarctica, a bioin-
formatic pipeline provides new insights into tularemia in Germany

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Francisella (F.) tularensis is a highly virulent, Gram-negative bacterial pathogen and the causative agent of the zoonotic disease tularemia.

A high quality circular genome sequence of the F. tularensis subsp. holarctica strain 12T0050-FLI was generated, analyzed and characterized. A novel bioinformatics pipeline could be established and evaluated.

Whole genome sequences from F. tularensis strains isolated in the years 2008-2015 in North-Rhine Westphalia, Germany, were generated. The phylogenetic analysis with different tools for genotyping revealed differences and allowed to determine a genotyping strategy for F. tularensis.

It confirmed the highly conserved nature of F. tularensis subsp. holarctica in Germany and was able to reproduce clades and subclades as determined using canSNPs with different approaches.

JP 06
Phenotypic and genotypic analysis of 68 Vibrio cholerae strains reveals various antibiotics resistances and suggests a potential missing link in the evolution of new serogroups

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Vibrio (V.) cholerae is a gram-negative, comma shaped proteobacterium inhabiting mainly standing waters in various aquatic environments. Toxigenic Vibrio cholerae strains, producing the phage-encoded cholera toxin CTX, mainly belong to serogroup O1 and O139. The agent is known to cause the life-threatening diarrheal disease cholera and therefore is of major public health concern in endemic countries.

Aim of this study was to investigate the genetic population structure of 68 toxigenic and non-toxigenic V. cholerae strains from various sources by the means of full genome sequencing (illumina®) and subsequent SNP (Single Nucleotide Polymorphism) typing. Furthermore, the resistance profiles of antibiotics relevant in cholera treatment were determined using Microdilution and Etest® according to CLSI guidelines.

Of the 68 strains investigated, 31 belonged to the toxigenic O139/O1 group and 37 strains to the non-toxigenic group. Within the toxigenic group various phage types were identified. Creating a minimum spanning tree based on SNP data of all toxigenic strains revealed a potential missing link in the evolution of serogroup O139 out of serogroup O1.

In phenotypic analysis, two strains of the collection were highly resistant to ampicillin. Notably, 20% of all investigated strains showed significant Cotrimoxazole resistance. Five strains with atypical antibiotic profiles were selected for further investigation towards the genomic origin of their resistances.

In summary, interpretation of whole genome data revealed a potential link between O1 and O139 strains, which to our knowledge has not been described so far and may be able to allow better understanding of the origin of serogroup O139 out of O1 in future studies. In a heterogeneous collection of V. cholerae strains from different sources we detected various phenotypic antibiotic resistances, encoded by genetic elements, commonly found in other gram-negative bacteria, suggesting interspecies genetic transfer.

JP 07
WITHDRAWN

JP 08
Francisella tularensis-macrophage interaction: A dual RNA sequencing approach

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As a gram-negative, aerobic, non-motile, non-sporulation small coccobacillus, Francisella tularensis (Ft) causes the zoonotic disease tularemia. Being highly infectious, this bacterium can infect more than 250 hosts from amoebae to mammals and can be transmitted to humans in various ways. These can be direct contact with sick animals or contaminated water or food, as well as tick, mosquito or fly bites or even inhalation. Ft subspecies (subsp.) tularensis (Ftt) is highly virulent and found in North America. Note, that Ftt is apprehended as a potential class A agent in bioterrorism since 2001. Ft subsp. holarctica (Fth) appear in both North America and Europe and these type B strains are mainly less virulent. So, by classical attenuation even live vaccine strains have been generated from type B strains. During infection, intracellular replication of Ft mainly occurs in macrophages (MΦs), but also dendritic cells, neutrophils, epithelial cells, fibroblasts and hepatocytes.

Vaccine candidate development against tularemia
until now is mainly ineffective in Ftt-challenged mice by means of protection or induction of sterile immunity. Therefore, new prevention and treatment strategies are required for this disease, but are aggravated by the limited knowledge of Francisella pathomechanisms. Developing a new vaccine against virulence factors might be a promising strategy to overcome actual limitations. Our project aims to detect such virulence factors of Ftt facilitating intra-MΦ replication and immune evasion as targets for the vaccine and therapy development against tularemia.

We established an infection model for THP-1 MΦs with a preferably low rate of apoptotic THP-1 MΦs with Ftt, moderate (Fth), and low (Fth, attenuated) virulent strains. Here, first data of dual RNA sequencing (dual RNA seq) are presented. This technique empowers studies on host-pathogen interactions, since it is perfectly suited to identify host factors regulated in a detrimental way by the pathogen as well as virulence factors involved in this regulation.

**JP 09**

**FOA-screening of genomes of Coxiella burnetii strains**

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From 2007 to 2010, the Netherlands had been confronted with the largest global Q fever outbreak ever, involving 4026 human cases. The Coxiella burnetii strain NL3262 was isolated during the Q fever outbreak and are clonal to Z3055, as they both contain the QpH1 plasmid, have the same MST33 genotype and have the same VNTR profile. Formal-order analysis (FOA) was used to study the similarity of the genome (chromosome and plasmid) of this strain with the genomes from other strains. Chromosomes from ten C. burnetii strains (Dugway 5J108-111; CbuK-Q154; Z3055; RSA 493; RSA 439; RSA439 clone 4; CbuG-Q212; RSA 331; NL3262; MSU Goat Q177) and eight plasmids (except CbuG-Q212 and Z3055) were studied. All reference genomes were imported from GenBank (https://www.ncbi.nlm.nih.gov/genome). Chromosomes and plasmids were studied with FOA tools such as ‘Map of genes’ and ‘Matrix of similarity.’ Pairs of numeric values of order characteristics from studied genomes and their components were mapped into pillars of dots on the MG. Components representing individual genomes are placed vertically, and some horizontal lines are formed with similar components in different genomes. The MS represents the similarity values for each pair of analysed genomes. Genomic similarity is determined by comparing the order characteristic values of their components. The MS toolkit enables one to obtain a list of only similar components of any pair of genomes. The MG tool showed that the chromosome of strain C. burnetii str. NL3262 distanced itself by the index of average remoteness (g) of 1.449640 from chromosomes of other strains (g 1.448295–1.448865). The MS was used for an advanced analysis of the obtained results. The complete similarity of the components of chromosomes and plasmids was determined by pairwise comparison. A total of 84.90% of the chromosomal components of C. burnetii strain NL3262 coincided completely with the chromosomal components of strain Z3055. For chromosomes of other strains, this percentage varied from 12.06% to 47.14%. The plasmid of strain NL3262 had 50.0% of the components being completely coincident with the components of the plasmid of RSA 331; with RSA 493 it was 29.89%. Thus, C. burnetii str. NL3262 is the closest to str. Z3055 by the similarity of the chromosomal components, but on the index of average remoteness of the chromosome and the similarity of the plasmids’ QpH1 components, it is the closest to strain RSA 331.

**JP 10**

**Whole-genome sequencing of Burkholderia pseudomallei isolate in Hungary**

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Burkholderia pseudomallei is the causative agent of melioidosis. The infection is endemic in Southeast Asia, Northern Australia, and Brazil. Melioidosis can be difficult to diagnose due to its varying clinical manifestations. Neurological cases occur rarely, the prevalence is only 3-4% from the total cases and the fatality rate is high without adequate therapy. The bacterium is commonly resistant to a wide range of available antibiotics. Here, we describe the complete genome sequence of the first imported Burkholderia pseudomallei case in Hungary. The strain marked as 584/OEK was isolated in 2008 from a 30 years old male patient with encephalomyelitis who travelled to India to work and contracted with the disease. The symptoms included weakness, fever, headache and facial-neuralgia developed three weeks later when he arrived back to Hungary. The strain was
isolated from blood culture. After the adequate therapy the patient fully recovered in spite of his serious symptoms. Whole-genome sequencing was performed using MiSeq Illumina platform (Illumina Inc., San Diego, CA, USA). For analysis we used a custom-made pipeline as follows: after quality control and trimming, the Illumina paired end reads data were de novo assembled with the Velvet. For annotation the RAST and PATRIC tools were used. The final assembly consisted of two chromosomes. The total genome size was 7, 2 Mb with 67, 9% GC content, the number of coding sequence was 6937. As an indication of antibiotic resistance OXA57 beta-lactamase was present. The whole-genome sequence was used to extract sequences for MLST analysis. We found that the strain belongs to a novel ST which was not previously recorded. The allelic profile of ST1643 was 1-12-6-4-1-8-87. Material and Methods: The sequence of the CRISPR cassettes contained repeats ranging in size from 20bp to 36bp, separated by spacers (from 20bp to 79bp). The greatest coincidence of spacers with protospacers of plasmids and phages was noted in CRISPR-cassettes 1 ("young") and 6 ("ancient"). They were specific for the bacteria of the family Alcaligenaceae, Enterobacteriaceae, Pseudomonadaceae, which are the causative agents of intestinal, opportunistic and severe nosocomial infections in humans. It was also noted that spacers correspond to protospacers of plasmids and plasmids of bacteria of the family Burkholderiaceae, Rhizobiaceae, Phyllobacteriaceae, pathogenic for plants, but not for humans.

**Results and Discussions:** This strain was used for research because it is widely used in worldwide biomedical research, retains its virulence and, according to the NCBI database, the CRISPR/Cas loci have not been identified in it (1). We identified six CRISPR loci in the CRISPR/Cas system of this strain, two groups of Cas genes characteristic of CRISPR/Cas systems type I and type III subtype III-A. CRISPR-cassettes measured from 541bp to 1751bp. The CRISPR cassettes contained repeats ranging in size from 20bp to 36bp, separated by spacers (from 20bp to 79bp). The greatest coincidence of spacers with protospacers of plasmids and phages was noted in CRISPR-cassettes 1 ("young") and 6 ("ancient"). They were specific for the bacteria of the family Alcaligenaceae, Enterobacteriaceae, Pseudomonadaceae, which are the causative agents of intestinal, opportunistic and severe nosocomial infections in humans. It was also noted that spacers correspond to protospacers of plasmids and plasmids of bacteria of the family Burkholderiaceae, Rhizobiaceae, Phyllobacteriaceae, pathogenic for plants, but not for humans.

**References:**

**JP 11**
WITHDRAWN

**JP 12**
WITHDRAWN

**JP 13**
Analysis of CRISPR/Cas system Mycobacterium tuberculosis H37Rv and screening of phages and plasmid through SPISER sites CRISPR-cassette

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At present, there is an increase in the number of patients with multidrug-resistant and extensively drug-resistant tuberculosis. The study of CRISPR/Cas-system Mycobacterium tuberculosis, directed at foreign nucleic acids using bioinformatics methods, will create the basis for developing new approaches to the diagnosis, prevention and treatment of this infectious disease. The aim of these studies was the study of the CRISPR/Cas system in the strain Mycobacterium tuberculosis H37Rv and the search and analysis of phages and plasmids via spacer sequences in CRISPR cassettes.

**Materials and Methods:** The sequence of the strain Mycobacterium tuberculosis H37Rv (NC-000962.3) was downloaded from the NCBI databases and analyzed using software for bioinformatics.

**Results and Discussions:** This strain was used for research because it is widely used in worldwide biomedical research, retains its virulence and, according to the NCBI database, the CRISPR/Cas loci have not been identified in it (1). We identified six CRISPR loci in the CRISPR/Cas system of this strain, two groups of Cas genes characteristic of CRISPR/Cas systems type I and type III subtype III-A. CRISPR-cassettes measured from 541bp to 1751bp. The CRISPR cassettes contained repeats ranging in size from 20bp to 36bp, separated by spacers (from 20bp to 79bp). The greatest coincidence of spacers with protospacers of plasmids and phages was noted in CRISPR-cassettes 1 ("young") and 6 ("ancient"). They were specific for the bacteria of the family Alcaligenaceae, Enterobacteriaceae, Pseudomonadaceae, which are the causative agents of intestinal, opportunistic and severe nosocomial infections in humans. It was also noted that spacers correspond to protospacers of plasmids and plasmids of bacteria of the family Burkholderiaceae, Rhizobiaceae, Phyllobacteriaceae, pathogenic for plants, but not for humans.

**References:**

**JP 14**
NGS of complete RNA virus genomes from original material and comparative analysis with the old approach of Sanger sequenced E-genes

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Tick-borne encephalitis is an emerging disease with central nervous system involvement and over 10,000 cases per year in Europe. The infectious agent, tick-borne encephalitis virus (TBEV), a flavivirus can be transmitted to humans via tick-bites. Over the last decades TBEV was detected and isolated from ticks in different Eurasian countries. For phylogenetic analysis the E-gene was amplified and Sanger sequenced. Since 2017 it is possible to utilise a NGS approach obtaining the whole viral genomes directly from the tick lysates. One question to address is whether the phylogenetic analysis will change if whole genomes are compared to E-genes and which analyses gives the most conclusive results.
To answer these questions we performed phylogenetic analyses using the whole genomes in comparison to the gene sequences to determine if they lead to the same information as the E-gene approach. Our results show, that investigating different gene selections often result in different phylogenetic patterns which we must carefully interpreted to find the best and most conclusive analysis method for the future.

**JP 15**
WITHDRAWN

**JP 16**
Epidemiology of *Bacillus anthracis* strains circulating in Italy based on 31-loci multi locus VNTR analysis

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**Introduction:** In Italy, anthrax is considered an endemic disease affecting ruminants with sporadic zoonotic occurrences in humans. Domestic and wild ruminants represent the most susceptible categories. The animal species bacterial agent has the characteristic of producing spores that can survive in the environment for several decades. Thanks to the considerable ability of spores to maintain viability and pathogenicity for many decades and thanks to its low costs of production, *B. anthracis* is considered one of the pathogens of greatest interest as a bacteriological weapon in a possible bioterrorist attack. In this investigation, we analyzed 222 *B. anthracis* strains (195 from animal species and 27 from environment) isolated in different Italian anthrax outbreaks from 1954 to 2017. The canonical SNPs assay (CanSNPs) and the multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA) have been used to differentiate the strains.

**Materials and Methods:** The phylogenetic identity 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. Eleven PCRs reactions were performed (2 singleplex and 9 multiplex) to amplify VNTRs.

**Results:** The analysis of 14 CanSNPs, allowed to identify four main lineages: A.Br.011/009, A.Br. 008/011, A.Br. 005/006 and B. Br. CNEVA. However, the lineage A major subgroup A.Br.011/009 (Trans-Eurasian or TEA group), represents the dominant population of *B. anthracis* in Italy, particularly in southern part of the country. The MLVA with 31 VNTRs analysis, demonstrated 55 different genotypes.

**Discussion and Conclusion:** The data obtained by 31 loci MLVA showed an increasing number of genotypes circulating in Italy, compared to the MLVA test at 15 loci by which we obtained just 30 genotypes. Most of them are genetically very similar to each other, confirming the hypothesis that all of them are the result of the evolution of a local common ancestral strain. The genotyping analysis with methods such as CanSNPs and MLVA, is a very valuable tool for studying the diversity, evolution, and molecular epidemiology of *B. anthracis*.

**JP 17**
Projected genotyping of *Brucella* spp. isolates, collected on the territory of Ukraine from 1945 to 2017

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**Background:** Brucellosis is a vicious worldwide zoonotic disease, caused by various species of the genus *Brucella*. In Ukraine human Brucellosis cases still occur each year. Molecular typing data, important for epidemiological outbreaks investigations or source tracking are missing.

**Project outline:** Since molecular typing data of Ukrainian Brucella isolates are not available, The National Scientific Centre "Institute of Experimental and Clinical Veterinary Medicine" (NSC-IECVM) has a unique collection of *Brucella* strains, isolated since 1945, which covers vast geographical parts of Ukraine. Around 50 of *Brucella* strains (*B. melitensis*, *B. abortus* and *B. suis*) in total will be typed using Multi-Locus Variable-Number of Tandem Repeat Analysis with 16 markers ( MLVA-16) and by core-genome based Mutation analysis (SNP-Typing). Genotyping of the collection can provide us with retrospect of brucellosis history in the Ukraine and evolutionary patterns of strains, reveal unique genome variants and improve the phylogenetic understanding of the genus *Brucella*.

**Material and Methods:** Bacterial isolates: The collection covers 4 *Brucella* species: *B. melitensis* (4 strains), *B. abortus* (72 strains), *B. suis* (15 strains) and *B. ovis* (46 strains). Samples were acquired in 11 regions from pigs, sheeps, cattle and humans. 50 of the samples are being constantly recultivated, the rest of the collection is stored in lyophilized condition under -4 °C . Genotyping: The species
affiliation of inactivated strains will be determined using the Bruce-ladder multiplex-PCR-approach (LopezGońi et al., 2008). MLVA genotyping will be performed according to, Le Flèche et al., 2006; Jiang et al., 2013; Scholz and Vergnau, 2013) using 16 microsatellite markers. Phylogeny of the Brucella species within Ukraine and including worldwide data will be implemented using BioNumerics version 5.1 software (Applied Maths, Belgium) (Zhi-Guo Liu et al., 2017). Clustering analysis will be performed using arithmetic averages (UPGMA) method, also maximum parsimony approach will be performed. Genotypes will be compared using the web-based Brucella MLVA database (http://mlva.u-psud.fr/).

For SNP typing (cgMLST) the commercially available software SeqShere (Ridom GmbH) will be used. **Conclusions:** With given collection, MLVA (and SNP) approach is a way to obtain a unique set of data for advanced understanding of the brucellosis epidemiology in Ukraine.

**JP 18**

Molecular biology research on the presence of *Francisella tularensis* strain in wild boar (*Sus scrofa*) samples

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*Francisella tularensis* is a highly contagious Gram-negative bacteria that causes tularemia or "rabbit fever" and it is contagious to humans. In 1911 *F. tularensis* was first described in Tulare County, California and occurs naturally in vertebrates, invertebrates, contaminated soil, water, and vegetation. This research was based on hunting samples (wild boar, *Sus scrofa*) harvested in January 2017 by Sanitary Veterinary and Food Safety Division Satu Mare from hunting and/or poached corpses.

The presence of *Francisella tularensis* was confirmed by molecular analysis. Targeted regions included the *ISFtu2* element, a insertion element-like sequence present in multiple copies, and the *tu4* gene, which encode outer membrane protein and are specific for *Francisella*.

*ISFtu2* allow the differentiation of *F. tularensis* by *Francisella*-like endosimulants/endosymbionts of host ticks such as *Dermacentor variabilis* and *D. occidentalis*. The choice of the insertion sequence-like element as a target for real-time PCR reactions is largely due to the increased sensitivity of this reaction due to its presence in several children/copies in the genome of different *Francisella* species and subspecies.

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**JP 19**

RNA-sequencing for transcriptome comparison between *Bacillus anthracis* and *Bacillus cereus* biovar *anthracis*

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The novel pathogen *Bacillus cereus* biovar *anthracis* (Bcbva) was isolated from wild great apes and other mammals that had died of an anthrax-like disease in rain forest areas of Côte d’Ivoire (strain CI) and Cameroon (strain CAM). Bcbva combines the chromosomal background of *Bacillus cereus* with the virulence plasmids pXO1 and pXO2 of *Bacillus anthracis* (Ba) and displays characteristics of both species on bacteriological level. Both, in *Ba* and Bcbva, expression of toxin and capsule genes are controlled by the pXO1-encoded global regulator AtxA and upregulated under host-mimicking growth conditions (presence of bicarbonate and CO₂). In non-anthrax strains of the *B. cereus* group, expression of virulence factors like phospholipases, proteases or haemolysins is controlled by the chromosomal regulator PleR. In contrast to *Ba*, where PleR is inactive due to a nonsense-mutation, the *pLcR* gene of Bcbva contains a frameshift in the 3'-part of the gene resulting in an altered C-terminus of the protein which is important for interaction with the co-regulator PapR. Thus, PleR-regulated genes are also inactive in Bcbva, which is confirmed by the lack of phospholipase C activity and haemolysis on blood agar plates.

The gene regulation of the investigated AtxA dependent virulence factors seem to be similar in *Ba* and Bcbva. However, previous analyses using reverse transcriptase PCR for selected genes indicated some differences. In order to get a more comprehensive view on the basis of global gene expression and to assess the significance of various virulence factors in the two species, RNA-sequencing was performed.
factors regarding pathogenicity, we started a comparative RNA-sequencing project on Bacillus cereus var. anthracis and classic Bacillus anthracis. RNA was extracted from strains grown under ambient and host-mimicking conditions, and libraries were prepared using standard protocols.

Preliminary analyses of the transcriptome data were performed using the TRAV software and confirmed the results obtained with reverse transcriptase PCR.

KP 01
Serbia – NATO cooperation in biodefence (Scientific monograph “Defence Against Bioterrorism”)

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Introduction: We published a book (Bioshield) that is based on a multidisciplinary approach towards biological threats that can, and have been previously used in bioterrorism attacks around the globe.

Methods: Current knowledge and evidence-based principles from the fields of synthetic biology, microbiology, plant biology, food science, forensics, tactics, infective medicine, preventive medicine, psychology, psychiatry, juristic and others are compiled to address numerous aspects and the complexity of bioterrorism.

Results: The main focus is on the levels of prevention against biological threats. Present synthetic biology as possible threat and proposals for protection. The Bioshield (book):

- explains top achievements in microbiology forensics,
- describes approaches towards panic management among people in case of a bio attack,
- analyzes how to recognize critical points in a food supply chain, and
- proposes corrective activities.

Strategy of deterring bioterrorism and intelligence strategies has been developed as well as problems that climate changes bring in the field of bioterrorism. It considered threat of natural focal fatalities and the latest achievements of quick and cheap diagnostics of the most dangerous infectious diseases. Examples from around the globe, along with the methodological approach on how to differentiate bioterrorism attacks from other epidemics are provided. However, epidemics are also discussed in the context of migrations, with the special emphasis on the current refugee migrations that affect not only Europe, but also the United States.

Conclusion: The Bioshield is of interest to experts from various fields of science as well as professionals working in the field. It encompasses examples and tools developed for easier, more specific and faster detection of possible bioterrorism threats, along with proposed actions for some aspects of a bioterrorism attack.

KP 02
New hazardous waste management regulation at LMA Laboratory Network in Georgia

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Laboratory of the Ministry of Agriculture (LMA) is a diagnostic laboratory that works on different animal infectious diseases including Especially Dangerous Pathogens. Laboratory network consists of 11 BSL2 laboratories: 3 Zonal Diagnostic Laboratories (ZDLs) and 8 Laboratory Support Stations (LSSs). Laboratory is staffed with trained personnel to handle Risk Group 2/3 agents. Due to the large amount of samples received and tested at LMA, accurate waste management is very important. In 2016-2017 LMA tested 810879 Brucellosis, 299 Anthrax and 499 Peste des Petits Ruminants specimens. In 2016 Georgian government promulgated a new regulation that required stricter control of hazardous waste generated from laboratories. Since 2016 LMA is in the process of interpreting the regulation, determining which requirements impact its operations. This study describes how LMA modified its existing waste handling procedures to make them compliant with the new regulation.

Methods and results: The label was developed to match existing hazardous waste procedures with requirements in the new regulation to identify relevant practices. A working plan was drafted on the basis of a risk assessment and reviewed by the LMA Biosafety Committee. Relevant chapters of the biosafety manual, biosafety SOPs, labels, signs and other waste related documents were modified.

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With respect to changes, specific annual trainings and workshops in biosafety were conducted for all laboratory staff. Special vehicles were equipped with GPS system.

The hazardous waste is being weighted before transportation. In 2017 approximately 9 tons of hazardous waste were transported from LSSs to Tbilisi and Kutaisi ZDLs.

Implementing the new waste management regulation required much work considering broad profiles of diagnostic tests done at the LMA laboratories. Central laboratory, ZDLs and LSSs have constant communication on biosafety issues and during waste transportation process. All procedures that fall under the description of waste management were done at a comprehensive manner in LMA network. The bio-waste management policy and plan established at LMA were documented in the biosafety manual and SOPs. The incinerators are used according to the international and local regulations and operated by certified persons. Effective hazardous waste management program is important for laboratory staff safety and significantly reduces negative impact of hazardous waste on public health and environment in future.

KP 03
WITHDRAWN

KP 04
EU Chemical, Biological, Radiological and Nuclear (CBRN) Centers of Excellence (CoE) initiative – Opportunities for regional collaboration

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The European Union (EU) Chemical, Biological, Radiological and Nuclear (CBRN) Centers of Excellence (CoE) Initiative was launched in May 2010 in response to the need to strengthen the institutional capacity of countries outside Europe to mitigate CBRN risks, including criminal activities, natural and accidental disasters. The objective of the CoE Initiative is to develop a structural, all-hazards CBRN policy at the national, 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; support national capacities to develop and enforce legal measures; ensure ownership and sustainability through an integrated regional approach; and provide a coherent package covering all aspects of CBRN proliferation, including safety and security, export controls, illicit transfers, emergency planning, crisis response, etc. The Initiative is very dynamic and evolving continuously as new partner countries inviting them to join the COE Initiative. For today there are 60 partner countries under 8 Regional Secretariats. The set-up of Regional Secretariats in different geographical areas and the designation of National Focal Points in partner countries have helped to create a flexible structure that should guarantee ownership and sustainability of the initiative.

One of the major strengths of the COE Initiative is the bottom-up approach for the elaboration of project proposals that represent the countries’ needs. The ownership of the process by the recipient countries is an asset that helps to ensure that the results achieved will be integrated into mainstream policies, legal systems, decision-making and administrative processes, and daily practice. However, there is a risk of duplication and the overburdening of partner countries. A mapping of the activities at the international and regional levels in the broader context of CBRN mitigation and coordination and implementation of collaborative projects would be beneficial both for partner countries as well as for international and national organizations working the regions.

KP 05
Official processes of approving genetic engineering operations with higher safety levels in enclosed genetic engineering facilities

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The German Genetic Engineering Law (GenTG), estimated in the early 90s, regulates the operation with genetically modified organisms (GMO). Besides deliberate release and placing on the market of products that contain or derive from GMOs, the law regulates the genetic engineering operations in enclosed genetic engineering facilities (including laboratories, manufacturing, greenhouses and animal facilities). The execution of the GenTG in Germany is divided up to the Federal Agency for consumer Protection and Food Safety (BVL) in Berlin and the respective responsible authorities of the federal states, whereas the Central Committee of Biological Safety (ZKBS) advises the federal government and the federal states on e.g. risk assessment of organisms or containment level assignment for genetic engineering operations. The federal states
are responsible for the approval of contained use in enclosed genetic engineering facilities and the supervision of these facilities. Moreover they monitor deliberate releases and placing on the market. The biosafety level in genetic engineering facilities (e.g. laboratories or animal facilities) is determined by the risk group of the respective organism and by the respective genetic engineering operations. These aspects lead to particular biosafety levels with the lowest level 1, up to the highest level of 4, if you work with viruses like the Ebola Virus. The bio-containment conditions for the respective levels are defined in the Genetic Engineering Safety Ordinance (GenTSV). By working with pathogenic agents which can cause low, moderate or high risks for humans or the environment, special containment conditions have to be applied. Especially operations with airways transferable agents need higher biosafety standards, e.g. the filtration of exhausted laboratory air by high efficient particulate filters, the obligatory work in biosafety cabinets class II or III, or special personal protection equipment. Evolving from risk assessment, the official procedures for the construction and operation differ widely. So far, for a genetic engineering laboratory with biosafety level 1 or 2 it is only needed to make an information, or a notification at the responsible authority in the federal state, whereas laboratories for projects rated level 3 or 4 have to be approved, in a process involving the BVL, considering structurally, technically or organizationally biosafety aspects of the facility.

**KP 06**  
**Spiez Convergence: A workshop series to discuss implications from current developments and technologies in the life sciences for arms control treaties**

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Research and technologies emerging from the life sciences bring beneficial aspects to our society but also unforeseeable risks regarding the biosafety and biosecurity. Many of today’s technologies have an inherent dual use potential. Spiez CONVERGENCE is a new workshop series organized by Spiez Laboratory, the Swiss Federal Institute for NBC - Protection. The workshop series intends to inform about latest advances at the intersections of chemistry, biology and associated research fields, as well as the adoption of such advances by the biotechnology and chemical industries, and to serve as a forum for discussion between stakeholders from academia, industry, arms control and policy making. The objective is to identify developments in chemistry and biology which may at some point have implications for the Biological Weapons Convention (BWC) or the Chemical Weapons Convention (CWC), and therefore may warrant further study. The BWC and the CWC are arms control treaties strongly linked to developments in science and technology. The increasing overlap between chemical and biological sciences, generally referred to as convergence in chemistry and biology, or short as convergence, has been noted by the treaties’ States Parties in recent conference reports where they recommended exploring its potential implications. Convergence describes an integrative and collaborative approach in the life sciences that brings together theoretical concepts, experimental techniques as well as knowledge of different disciplines at the crossroads of chemistry and biology. The series is designed as a Swiss contribution to a science and technology review with the aim to support the implementation of the Conventions by informing scientists, policy makers, and arms control specialists about novel developments in the life sciences that might be related to dual use research (of concern) and to enhance the communication between the science and the policy world for supportive regulatory decision making for such scientific developments.

**KP 07**  
**DURC – An unclear threat in a complex security environment**

J Opper and G Jeremias  
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In the past decade, Dual Use Research of Concern (DURC) has come up as one potential threat stemming from research in the life sciences. DURC denotes research that can be directly misused by a malevolent actor, thus making it especially suitable for terrorist attack as well as for state sponsored bioweapon programs. This has caused a widespread call for tighter regulation and oversight of very risky research projects through governments and organizations of academic self-governance. Much has been written about DURC in the past and currently there is a high number of position papers, proposals, studies, etc. in circulation that recommend policy measures for DURC regulation. At the same time, these papers try to define what DURC actually is, e.g. what kind of research falls under a DURC-definition and which does not. However, there seems to be no consensus on what falls under the category of DURC research and how such research should be regulated. This confusion...
is closely linked to differing ideas about which is
the object of security concern within the different
approaches to DURC governance.

The poster will present preliminary findings from a
qualitative study analyzing the different positions
taken by the literature on DURC regarding the
different notions of security as well as the objects
that need to be secured by DURC regulations. The
poster will show, that within the literature we find
different understandings security (human vs. state
centric), of DURC (material vs. immaterial) and
different foci regarding the threat stemming from
DURC (symmetric, asymmetric, hybrid), leading to
misunderstandings and an incoherent policy frame-
work.

The poster will clarify some of these misunderstand-
ings and contribute to the establishment of a more
precise understanding of DURC thus contributing
to the establishment of a comprehensive policy frame-
work.

**KP 08**

**Strengthening risk analysis – Including socio-political factors into scenario**

J. Opper, H. Martin, and T. Stegmaier

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Risk analysis plays an important role in preparing
for possible speared of biological agents. Consciously
or not, theses risk analyses make use of scenarios,
insofar as they try to predict the likelihood and
possible consequences of a future event.

While traditionally risk analysis techniques focus on
technical data gathered though rather quantitative
methods to capture risk, it is the argument here,
that by using scenarios also socio-political factors
play an important role in predicting future events.
Thus, risk analysis is more complex than often taken
into account.

Current and future events do not play out in an
empty space, rather they are embedded in a complex
social, political, cultural and economic environment.
The poster will take a closer look at socio-political
factors, which provide an environment in which
the future events play out. Socio-political factors
are of special importance because usually official
institutions react to the events in order to decrease
damage caused by an event. By taking mitigating
strategies into account, risk analysis, often implic-
ily makes assumptions about the future political
environment in which institutions might or might
not adopt certain policies.

Here, socio-political factor contribute immensely
to decision making by state and non-state actors.
However, implicit or explicit assumptions about
which decisions an actor might make, are based
primarily on assumptions about the socio-political
environment in which the future event takes place.
Therefore, risk analysis always includes such
assumptions.

These assumptions underlying the model must be
made clear and if possible should be supported
on facts and state of the art research. The poster
presents a conceptual approach to risk analysis
and makes the case for a more explicit role of the
social sciences in risk analysis. Research coming
from the social sciences can make an important
contribution to risk analysis by providing a base for
the underlying assumptions.

**KP 09**

**Constructive simulation in management of biological attack consequences**

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The analysis of the current risks and threats on
the European continent require the establishment
of the capable infrastructure for a quickly reacting
to a bioterrorist attack because the bioterrorist
attack can cause a major biological crisis. Any ac-
tion aimed at increasing the reaction and response
capacity in the event of a biological incident is
welcome.

In order to make an informed and substantiated
decision on how to respond to bioterrorist threats
and how to resolve situations dynamically, Military
Medical Research Center specialists in collaboration
with 'war games' specialists from War Gaming and
Doctrinal Experimentations Center, used construc-
tive computer simulations experiments for assessing
bioterrorist threats, vulnerabilities and risks.

*Bacillus anthracis* has been chosen for being used
in the constructive simulation experiment, because
it is known as the biologically bacterial agent most
used for possible biological attacks.

The *scenario* of the experiment was fictional and
involved the simulation of several biological and
bioterrorist attacks on urban areas with various pop-
ulations and densities, in which military units are
deployed. The evaluation of the logistic response in
the fictional biological attack was based on the data
provided by the computer constructive simulation
experiments.

Knowledge and awareness of situations that may
arise in such a hypothesis is extremely important for
assessing the evolution and accurately estimating
the need for forces and means for intervention before
and after the biological attack.

The experimental model for defense exercises against the effects of the biological weapons and of bioterrorism is reliable and can be adapted for application in situations of massive contamination with biological agents.

**KP 10**

**Biosecurity implications of CRISPR/Cas-based genome editing**

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Recent advances in genetic engineering have led to the development of genetic tools including CRISPR/Cas9 which allow genome manipulation with previously unthinkable precision and efficiency – at least in theory. In 2016, the Worldwide Threat Assessment of the US Intelligence Community stated that genome editing “probably increases the risk of the creation of potentially harmful biological agents and products” [1]. Therefore, genome editing tools are classified as enabling techniques for the development of biological weapons of mass destruction. In history, there was a great interest in the use of genetic engineering for the generation of modified biological warfare (BW) agents as exemplified by the Soviet Union’s large-scale BW programme [2]. Today, non-proliferation measures aim at mitigating the misuse of genetic engineering for hostile purposes. Ideally, this should also include a thorough risk assessment of novel genome editing tools based on experimental findings. Here, we provide an overview of different naturally occurring types of CRISPR/Cas systems and highlight some applications in biomedicine and basic research. Furthermore, we analysed the genomic organisation and gene activities of an endogenous CRISPR/Cas operon using *Burkholderia glumae* as model organism [3]. Combined with further studies of regulatory mechanisms and immune defence capabilities, this will allow us to perform efficiency testing of genome manipulations in *B. glumae* by the use of commercially available CRISPR/Cas kits and plasmids. Finally, we address the question how to access potential biosecurity threats emanating from genome editing techniques in a systematic approach.

**References:**


**KP 11**

**A peer review visit exercise at the Richard Lugar Center in Tbilisi, Georgia**

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The Biological and Toxin Weapons Convention (BTWC) is a cornerstone of the international community’s efforts to address the proliferation of weapons of mass destruction and contributes to the promotion of biosafety and biosecurity on a global level. Compliance with the BTWC requires a state party to ensure that no life science activity – research or otherwise – that is contrary to peaceful purposes takes place anywhere within their jurisdiction. Up to this day, there is no legally binding mechanism by which the compliance of any state party with this requirement can be confirmed. Voluntary transparency visits are therefore a useful and important means of demonstrating compliance to others and building confidence.

Having observed the successful conduct of such a visit at the Bundeswehr Institute of Microbiology in Munich, Germany in 2016, the Republic of Georgia has decided to host a similar event at the Richard Lugar Center in Tbilisi with a level of transparency equaling that of the 2016 German visit. The Federal Republic of Germany, upon request by the Georgian government, has agreed to share their experiences and assist in the preparation of the Georgian Peer
Review Exercise. To this end, multiple preparatory visits to the Lugar Center have been conducted by representatives of the German Federal Foreign Office, the Bundeswehr Institute of Microbiology and the Bundeswehr Verification Center.

After successful preparations, the visit will now definitely be conducted in November 2018, providing representatives from a wide spectrum of BTWC states party the opportunity to observe firsthand that there is no evidence for any activity contrary to peaceful purposes being conducted at the Richard Lugar Center, Tbilisi.

This exercise further amplifies the momentum of taking the Peer Review concept through a strengthening and expansion process that was started by Germany in 2016. For the third year in a row (after the German exercise and a Peer Review Visit hosted by the Kingdom of Morocco in 2017), actual life science experts from around the globe will be able to assess BTWC compliance by going into the laboratory rooms, examining equipment and interacting with on-site personnel. All three exercises show that it is clearly possible to reconcile a level of transparency previously thought impossible with the legitimate security and intellectual property interests of the visited facility.

**KP 12**

The European Mobile Laboratory Consortium (EMLab) as partner of WHO-GOARN, WHO-EDPLN and the European Medical Corps (EMC)

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The EMLab was established in the framework of IFS 2011/272- and funded by the EC Development and Cooperation office (DevCo). The project lasted from the end of 2011 to the end of 2015 and comprised partners from Europe and Sub Saharan African Countries. Three mobile laboratory units for diagnostics of high risk pathogens were established and a pool of scientists was trained for deployment in case of infectious disease outbreaks. Main technical implementing partners were the Bundeswehr Institute of Microbiology in Munich, Germany (lab units and training), the Lazzaro Spallanzani National Institute for Infectious Diseases in Rome, Italy (training and focal partner NIMR Tanzania), the focal Partners in Africa, Institute for Lassa Fever Research and Control at the Irrua Specialist Teaching Hospital (ILFRC-ISTH) in Nigeria and the National Institute for Medical Research (NIMR) of Tanzania, while the project was coordinated by the Bernhard Nocht Institute for Tropical Medicine in Hamburg, Germany. EMLab, under WHO-GOARN and with the input from all the partners of the consortium, like the Robert Koch Institute in Berlin, Public Health England in Porton Down, Philipps University in Marburg, INSERM-P4 Lyon, and Spiez Laboratory in Switzerland, provided molecular diagnostics in the Ebola outbreak in West Africa from March 2014 to September 2016. Since July 2016 EMLab is registered in the newly (February 2016) established European Medical Corps (EMC) as part of the voluntary pool of the Union Civil Protection mechanism and has been deployed two times since to infectious disease outbreaks in Africa, and has participated in European exercises for civil protection and humanitarian aid. In July 2016 one EMLab unit was deployed to the Yellowfever outbreak in the Democratic Republic of the Congo and in November 2017 one lab unit was deployed to the quickly contained outbreak of Marburg Virus in Uganda. In April 2017 EMLab participated at a MODEX field exercise where a lab unit was deployed to a complex disaster scenario and in May 2017 EMLab took part in a MODEX table top exercise.

EMLabs' operational readiness and maintenance is currently funded by the Global Health Protection Program of the German Federal Ministry of Health. Deployments in case of infectious disease outbreaks are funded by the German Federal Foreign Office and co-funded by the Directorate General for Civil Protection and Humanitarian Aid Operations of the European Commission (DG-ECHO).

**KP 13**

Promoting scientific transparency to facilitate the safe and open international exchange of biological materials and electronic data

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Scientific communication, collaboration and progress are enhanced through the exchange of data, materials and ideas. Recent advances in technology, commercial proprietary discovery and current local and global events (e.g., emerging human, animal and plant disease outbreaks) have increased the demand, and shortened optimal timelines for material and data exchange, both domestically and internationally. Specific circumstances in each case, such as the type of material being transferred (i.e., Select Agent, disease-causing agent and assessed biosafety risk level) and current events, dictate the level of agreements and requirements. Recent lessons learned from emerging disease issues and emergencies have demonstrated that human engagement and increased science diplomacy are needed to reinforce and sustain biosafety and biosecurity practices and processes for better scientific transparency and biological threat reduction. A reasonable and accepted framework of guidance for open sharing of data and materials is needed that can be applied on multiple cooperative levels including global and national. Although numerous agreement variations already exist for the exchange of materials and data, regulations to guide the development of both the language and implementation of such agreements are limited. Without such regulations, scientific exchange is often restricted, limiting opportunities for international capacity building, collaboration and cooperation. International case histories will be presented that illustrate the complex nature of scientific exchange. Recommendations are made for a dual bottom-up and top-down approach that includes all stakeholders from beginning negotiation stages to emphasize trust and cooperation. This approach has a broader aim to increase international scientific transparency and trust in a safe and open manner supporting increased global one health security.

NP 01
Global Partners in Biosecurity

Serological and molecular biological detection of West Nile virus in the cerebrospinal fluid of patients of the Military Hospital of Tunisia, 2012-2017

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Introduction: West Nile Virus (WNV), an enveloped, single-stranded, positive-sense RNA virus of the *Flaviviridae* family, is transmitted by mosquitoes and primarily infects birds but occasionally also humans and horses. Several outbreaks of West Nile virus infections in humans have been reported in Tunisia during the last two decades. These outbreaks have been associated with a higher incidence of severe diseases, although 80% of human WNV infections are asymptomatic. Most symptomatic patients experience an acute systemic febrile illness; less than 1% of infected patients develop a neuro-invasive disease, which typically manifests as meningitis or encephalitis. The aim of this study is to evaluate the prevalence of WNV in cerebrospinal fluid (CSF) samples of the Military Hospital of Tunis from the last 6 years.

Material and methods: In the present study, about 300 CSF samples, collected from patients hospitalized in diverse services of the Military hospital of Tunisia from 2012 to 2017, were screened by a WNV IgM enzyme-linked immunosorbent asay (ELISA). To confirm ELISA positive samples, they were subsequently analyzed by real-time PCR assays for WNV. For this purpose, two real-time PCR assays targeting WNV lineage 1 and 2 strains respectively were established and validated on the Magnetic Induction Cycler (MIC).

Results: A total of 1500 human CSF samples will be included in the study. 300 out of these 1500 patients were shown by cytological examination of the CSF to have increased leucocytes numbers (n > 10): 47% women and 53% men. The mean age of these 300 patients was 32.9 years.

One WNV-positive sample was detected so far by IgM ELISA and could be confirmed by real-time PCR for WNV lineage 1 - a group of WNV circulating in Europe, the Mediterranean region, in Asia and Australia.

Conclusion: Preliminary results have shown that WNV infections can be detected in CSF samples serologically by IgM ELISA as well as molecular biologically by real-time PCR. The establishment of two real-time PCR assays for the detection of WNV lineage 1 and 2 usable for human CSF, improves the rapid diagnosis of a re-emerging global pathogen that remains an important public health challenge.
NP 02
Real-time PCR detection of *Brucella* in the cerebrospinal fluid of patients of the Military Hospital of Tunisia, 2012-2017

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Introduction: Brucellosis, the most common zoonotic infection worldwide, is caused by the bacterial genus *Brucella*. Globally, the organism causes over 500,000 infections yearly. Neurobrucellosis (NB) is a rare complication of this zoonotic infection which occurs in less than 5% of cases of brucellosis. 32 cases of NB were identified in the south of Tunisia between 1999 and 2010. The aim of this study is to evaluate the prevalence of NB in patients of the Military Hospital of Tunisia diagnosed with encephalitis and meningitis during the last 6 years.

Material and Methods: After the establishment and validation of a real-time PCR assay for *Brucella* species on the Magnetic Induction Cycler (MIC), a retrospective study over a 6-year period (January 2012 to December 2017) was conducted. Cerebrospinal fluid (CSF) samples collected from patients hospitalized in diverse services in the Military hospital of Tunisia will be screened for NB molecular biologically.

Results: A total of 1500 CSF samples will be included into the study. 300 out of these 1500 patients were shown by cytological examination of the CSF to have abnormal leucocytes numbers (n > 10): 47% women and 53% men. The mean age of these first 300 patients was 32.9 years.

A positive result was detected using Brucella real-time PCR in three patients so far. Subsequent new generation sequencing (NGS) of those three real-time positive samples will be performed to confirm the results.

Conclusion: The diagnostic of NB is difficult because its clinical manifestations are non-specific and the sensitivity of routine culture tests is low. This study demonstrates the power of real-time PCR that may become a standard method for diagnosing NB. This molecular biological method could be useful for rapid diagnosis of NB, especially since the Centers for Disease Control and Prevention (CDC) has declared Brucellosis as one of the major Category B bioterrorism agents.

NP 03
Establishing diagnostic *Bacillus anthracis* PCR at the National Center for Disease Control and Public Health (NCDC) of Georgia

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*Bacillus anthracis* is a worldwide spread pathogen and the causative agent of the zoonotic disease Anthrax. It is a gram-positive, rod-shaped bacterium, with circular DNA and 2 plasmids: pXO1 and pXO2. The plasmids encode 3 virulence factors: protective antigen PA, edema factor and lethal factor. Anthrax is a common disease in domestic livestock and wild animals; but can also have a big impact on public health.

Quantitative real-time PCR (qPCR) is a main method in molecular testing, especially in the area of clinical diagnostics, because of its high sensitivity and specificity and low turnaround time. Although commercially available qPCR assays have high usage in detection, quantification and typing of different microbial agents, it is essential for many laboratories to create and validate in-house qPCR assay. The algorithm of qPCR diagnostics for *B. anthracis* contains 3 main components. The first step is the detection of the chromosome via *dhp61* gene followed by detection of the plasmid pXO1 via the *pagA* gene and of pXO2 via *capC*. Finally *B. cereus* species can be detected by screening for *gyrA* (in case chromosomal *dhp61* is negative, but plasmids are positive and might have been transferred). As a result we will show validation and verification procedure of the *Bacillus anthracis* qPCR at the National Center for Disease Control and Public Health (NCDC) based on the protocol of the Bundeswehr institute of Microbiology (BwIM). For validation of the qPCR specially designed plasmids (containing *dhp61*, *pagA*, *capC* and *gyrA* fragments) were used as positive controls. All other reactions and statistics were performed at the NCDC. The main benefit of the new protocol is high sensitivity, which means that it was possible to reach a low limit of detection (LOD) with 4-6 molecules in one reaction.

In summary we have verified the in-house qPCR assays for all 4 targets from the BwIM at the NCDC for the first time. The work was part of the Georgian-German Partnership Programme “Establishment of a Western Asian Network for the Improvement of Biosecurity in the Caucasus Re-
The Role of a Tunisian Reference Microbiology Lab in the rapid diagnosis of potentially epidemic emerging infectious diseases

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Over the last decades, an increasing incidence and transboundary spread of emerging infectious diseases have been noted worldwide. To minimize the health and socioeconomic impacts of these diseases, major challenges must be overcome on national and international levels. In each country, the implementation of an optimal national system for the prevention and management of biological hazards is mandatory. In this system, the diagnostic laboratory plays a central role by identifying the etiological agent causing an outbreak, and by providing timely and accurate information required to guide control measures. In Tunisia, the microbiology laboratory of Charles Nicolle Hospital of Tunis, is the national reference laboratory for HIV, respiratory viruses including influenza, measles and rubella (serology), and is the National Research lab for antimicrobial resistance. The microbiology laboratory of the Charles Nicolle Hospital was therefore chosen by the Tunisian Ministry of Health to participate in the “German Biosecurity Programme” in cooperation with the Robert Koch Institute (RKI) and the German Agency for International Development (GIZ). This program supports the development of a harmonized Tunisian system for the prevention and management of biological hazards that meets international scientific and regulatory requirements. The main objective of this collaboration is the reinforcement of laboratory capacities in two directions: i) the implementation of rapid diagnostics of potentially epidemic highly pathogenic viruses and bacteria by advanced molecular techniques and ii) biosafety and biosecurity by supporting laboratory training and quality assurance. Therefore, the laboratory will be able to detect cases of a broader range of highly pathogenic agents following GLP in order to support outbreak investigations and inform the Ministries of Health and Defense in a timely manner. In conclusion, training in diagnostic techniques and biosafety measures as well as upgrading technically and logistically the lab and epidemiological capacity in Tunisia is critical to minimize the impact of future emerging infectious disease epidemics.

NP 05
Phylogenetic analysis of Ukrainian Bacillus anthracis strains

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Background: Anthrax is a widely spread zoonotic disease which poses a serious threat to public and animal health. Sporadic cases of anthrax occur each year in Ukraine both among farm animals and humans. Cutaneous form of anthrax is the most widespread in Ukraine. The capability of Bacillus anthracis spores to remain viable in soil for decades, as well as the possibility to use this pathogen as biological terror agent make effective diagnostic and research capabilities extremely important. This comprises bioforensic capabilities including state-of-the-art methods for accurate genotyping of B. anthracis strains.

Materials and methods: A total of 12 B. anthracis DNA samples from Ukrainian strain collection at the State Control Institute of Biotechnology and Microorganism Strains (Kiev, Ukraine) were studied at the Bundeswehr Institute of Microbiology (Munich, Germany) by qPCR to confirm the presence of anthrax genome and plasmids. To characterize regional and global phylogeographic patterns of these strains, canonical Single Nucleotide Polymorphisms analysis (canSNP) and Multiple-Locus Variable-number of tandem repeat Analysis (MLVA-31) were conducted. MLVA tree was built using Bionumerics software.

Results: B. anthracis chromosomal DNA-markers as well as those of the pXO1 plasmid could be detected in all 12 DNA samples. However, only 5 out of 12 tested strains contained the pXO2 plasmid-marker. In the further process of B. anthracis DNA genotyping using SNP-analysis, we found that all tested pXO2 positive strains group into the A.Br.008/009 SNP-clade, which belongs to the major “A” branch of B. anthracis. This clade is also known as Trans-Eurasian subgroup, which is spread across Europe, the Middle East, and parts of Asia, including China. MLVA-31 analysis suggested that group of Ukrainian B. anthracis genotypes was closely related to strains from Southern Europe (in particular, to Bulgarian, Greek and Italian isolates). The preliminary results indicate that the pXO2-negative strains might be related to Russian vaccine strains.

Conclusions: The infrequent occurrence of anthrax in the country of Ukraine is likely caused by a heterogeneous population of B. anthracis. This
population is phylogenetically composed of at least two different canSNP groups of the world-wide dominating A-branch of the pathogen. While one group might stem from environmental recovery of live vaccine strains used in Ukraine (or the former Soviet Union in the past) the other one, A.Br.008/009 is likely the one that could be enzootic as indicated by the presence of related strains in countries of southeastern Europe in relatively close geographical vicinity to Ukraine.

**NP 06**

Crimean-Congo hemorrhagic fever virus in ticks in Kosovo, 2014-2016

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Crimean-Congo hemorrhagic fever virus (CCHFV) poses a serious threat to human health in Kosovo. CCHFV is transmitted to humans through tick bites or smear infection, crushing of infected ticks or direct contact with blood or tissue of infected animals or humans, including nosocomial transmission. Therefore we sampled 1815 ticks collected from domestic animals and the environment. Ticks were collected in the years 2014/2016 and tested for viral RNA of CCHFV by RT-PCR. In the study were found the following tick species in Kosovo: *Hyalomma marginatum* 764 (42%), followed by *Rhipicephalus bursa* 508 (28%), *Ixodes ricinus* 429 (23.6%), *Dermacentor marginatus* 95 (5.2%) and *Haemaphysalis* spp. 40 (2.2%). Overall, CCHFV RNA was detected in 14 ticks (0.77%). Twelve out of 508 R. bursa ticks (2.36%) collected from the cattle, sheep and goats in the eastern part of Kosovo resulted positive on CCHFV (in nine R. bursa specimens from the Prishtina region, as well as in three from the Gjilani region) and two out of 764 H. marginatum ticks (0.26%) collected from cattle of Malisheva municipality in the central and southwestern part of Kosovo. Our study confirmed that CCHFV is circulating in Kosovo mainly in R. bursa and H. marginatum ticks, however in lower prevalence than in the previous studies from the year 2012. The low prevalence of CCHFV in ticks during the years 2014/2016 correlate with the small number of infected persons with CCHF in Kosovo and the circulating of the CCHFV (AP92-like strains) in R. bursa ticks, which is associated with its small pathogenicity or is apathogen for humans.

**NP 07**

Development and implementation of GIBACTH like model for emerging infections and outbreak management in Karachi Division Province Sindh Pakistan

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Emerging infectious diseases pose a threat on global health security and cause economic impact related to unexpected morbidities and mortalities. For the management and prevention of outbreaks caused by the intentional or unintentional release of emerging infections, low and middle income countries (LMIC) require increased numbers of skilled health care workers (HCW).

Avoiding the threat of nefarious use of agents on human or animal populations is the highest priority for global security. Working with partner countries, GIBACTH has focused to develop the capacity building of public health personnel (epidemiologists, microbiologists, veterinarians, lab personnel and equivalent life sciences) of LMIC regarding biosafety, biosecurity, outbreak investigation, infectious disease surveillance and communication.

From Pakistan, so far 8 participants have been selected for GIBACTH. All are frontline HCW of the government health care system and are involved in disease surveillance, and outbreak investigation and management. They are using acquired knowledge in their work and also sensitise the decision makers regarding biosafety and biosecurity when and where required.

**Rationale:** Pakistan faces a scarcity in trained HCW to deal with threats associated with intentional or accidental releases of emerging biological agents. There is urgent need to build a core group of professionals, who have sound knowledge, training and experience to deal with these threats. We are proposing a conceptual plan to implement a GIBACTH-like model in the Karachi division (18 towns, 3.5 million population), province Sindh by using resources from the department of health and GIBACTH. The objective is to build capacity of frontline HCW regarding biosafety, biosecurity, surveillance, and outbreak investigation and management.

**Methodology:**
1. Identification and enrollment of frontline HCW...
2. Conduct 3 workshops of 5 days in the conference hall at Karachi
3. Modification of teaching material according to country needs
4. Trainers and facilitators will be selected out of the GIBACHT alumni
5. GIBACHT will provide the methodological support if possible
6. A certificate will be awarded to the successful candidate

**Expected outcome:** Provide human resource trained in biosafety and biosecurity, surveillance, outbreak investigation and management

**Way forward:** Implementation of same model in other four division of Province Sindh

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**NP 08**

**German-Georgian network for the improvement of biosecurity in the Caucasus Region**

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A German-Georgian joint project between the National Center for Disease Control and Public Health (NCDC) and the Bundeswehr Institute of Microbiology (IMB) has been conducted since 2013 under an agreement between the governments of Germany and Georgia. Our collaboration intends to expand the German-Georgian network, which was established in the first project phase from 2013 to 2016. During the past few years German and Georgian institutions have been able to conduct a number of activities in the field of biosafety and biosecurity. Most of them have been operated at the Richard G. Lugar Center for Public Health Research (CPHR) of the NCDC, which is the primary institution for diagnostics of infectious diseases in Georgia and the main collaboration partner of the German Biosecurity Programme. Within the project German scientists initiate practical workshops for detection and handling of dangerous infectious pathogens, trainings on biosafety aspects, field studies and intensive trainings in modern laboratory methods. The IMB implemented a training program for Georgian scientists to cover education and training in modern molecular diagnostic methods, cell culture and immunological detection methods as well as state-of-the-art diagnostic assays.

In the new funding period it was possible to start collaboration with the National Food Agency of the Ministry of Agriculture and conduct a joint field study to collect and investigate tick and cow sera samples. In 2017 we held a Biosafety and Biosecurity Symposium in the framework of the German Georgian year in Tbilisi to celebrate our strong German-Georgian partnership. The symposium featured talks of biosafety and biosecurity experts from different institutions and ministries regarding the main achievements over the last years and current biosafety and biosecurity topics.

Georgia is working together with partner countries to support Global Health Security and is playing an important role for improving biosafety and biosecurity in the south Caucasus region. In order to increase the ability of local first responders to prevent and respond effectively to public health emergencies the country has to develop appropriate capacities and maintain countermeasures against biological threats. The German-Georgian collaborative project contributes to these efforts and strengthens NCDC’s abilities to respond to potential biological threats and control current and future disease outbreaks in Georgia.

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**NP 09**

**Cluster of human cases of tick-borne encephalitis transmitted by tick biting in Georgia**

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The tick-borne encephalitis virus (TBEV) occurs worldwide and is endemic in Eastern and Central Europe, Far East and other parts of Asia, creating nearly 20,000-30,000 natural foci from Europe to Japan. Certain countries, including Georgia, have not yet been included in the search of the viruses’ presence and distribution and therefore leave an empty space on the TBEV prevalence map.

Within the framework of the German-Georgian
Improvement of laboratory diagnostics of leptospirosis and investigation of predominant serovars endemic in the country of Georgia

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Leptospirosis is a zoonotic disease caused by spirochetes of the genus *Leptospira* and affects humans and a wide range of domestic and wild animals. The formal registration of leptospirosis in the country of Georgia began in the 1950s. Collected epidemiological data suggest that the infection is widely distributed throughout the country. The course of the disease is frequently severe with gradually increasing tendency and duration. Water is mainly implicated as a risk factor in the infection transmission. Since 2006 a significant increase of leptospirosis in Georgia has been observed. The highest incidence was reported in 2017 with 2.02 (75 cases) per 100 000 population, including 7 lethal cases. Young and middle aged people are mainly exposed to leptospirosis in Georgia. The disease is spread with equal intensity in urban as well as rural areas.

Current routine laboratory diagnosis of leptospirosis in humans in Georgia is limited, and performed only by screening with a commercially available *Leptospira* IgM and IgG ELISA. Moreover, only little is known about present serovars in the country. Within the framework of the German Biosecurity Programme we initiated a study to investigate key questions about diagnostics of leptospirosis in animals and humans in Georgia. Our research activities include identification of prevailing serovars of *Leptospira* in the country and the evaluation of reservoir-human serotype relationship.

An onsite training of *Leptospira* microscopic agglutination test (MAT) technique was provided by the specialists of Bundeswehr Institute of Microbiology (IMB) in October, 2017 at R. Lugar Center of the NCDC in Georgia. Afterwards, MAT testing for antibodies demonstration against specific serovars of human (preferably ELISA positive) sera, was implemented. A panel of 24 serovars is currently used for MAT testing. During a field study planned in October 2018, kidney tissue samples collected aseptically from rats will be cultured for *Leptospira*. For further investigations we will develop qRT-PCR diagnostics for recognition of *Leptospira* strains belonging to the intermediate group (less pathogenic group).

Implementation of new diagnostic techniques and improvement of existing methods will significantly enhance the capabilities for detection of leptospirosis infections in Georgia. It will provide medical and public health professionals with more valuable diagnostics and epidemiological information about emerging serogroups of *Leptospira*.
NP 11
Implementation of the Tunisian Taskforce against biological threats: A Tunisian - German cooperation project

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The Tunisian Taskforce against Biological threats was created in the framework of the Tunisian-German cooperation in the field of biological security. It responds to an urgent need to strengthen the cooperation between health and security authorities for readiness and preparedness to biological threats caused by highly pathogenic or bioterrorism-related agents. The cooperation project aims to train and equip Taskforce responder units in order to enable them to take immediate action when a bio-incident occurs. It shall also facilitate the establishment of a Tunisian contingency plan against biological threats.

The project is implemented in close collaboration between the Tunisian General Directorate of Military Health (DGSM) and the Robert Koch Institute (RKI) with support of the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH. Further selective support is provided by the German Federal Criminal Police Office and the Federal Police. The project started in early 2017 and currently runs until the end of 2018. It is commissioned and financed by the German Federal Foreign Office.

On the Tunisian side, the ministries of Defense, Public health and Interior through its two branches the Technical Police Division and the civil protection are involved in the project. Members of the Taskforce units are also experts from these ministries: A medical physician as a team leader, a CBRN expert, a bio agent for onsite sampling and a forensic police officer for evidence collection.

Two major milestones of the project have already been achieved. The Tunisian Biological Taskforce was created, consisting of three main interdisciplinary responders units with four members each. By means of practical and theoretical workshops these 12 experts were equipped and trained on the use of personal protection equipments (PPE), sampling of biological and forensic evidence on a potentially contaminated crime scene, decontamination and the use of communication systems.

Moreover, an inter-agency and inter-disciplinary cooperation network in Tunisia on exceptional biological threats was established.

The future vision for the sustainability of the project is to continue training the taskforce units as well as to widen and structure the inter-agency cooperation in the framework of a national contingency plan against biological threats.

NP 12
Novel ELISAs for the sensitive and specific detection of Crimean-Congo Hemorrhagic Fever Virus (CCHFV) - specific antibodies in human and animal sera

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As the most widespread tick-borne arbovirus causing infections in numerous countries in Asia, Africa and Europe, Crimean-Congo Hemorrhagic Fever Virus (CCHFV, family Nairoviridae) was included in the WHO priority list of emerging pathogens needing urgent R&D attention. To ensure preparedness for potential future outbreak scenarios of this zoonotic pathogen, reliable diagnostic tools for identification of acute cases as well as for performance of seroprevalence studies are necessary. In this study, we employed our patented immune complex (IC) ELISA technology for the development of novel ELISA tests detecting CCHFV-specific IgM and IgG antibodies in both human and animal sera.

Tests intended for use on human samples were validated using a serum panel comprising longitudinal samples collected in Kosovo during the years 2013-2016 from 15 patients with an RT-PCR-confirmed CCHFV infection. All assays exhibit high reproducibility (low intra-/inter-assay variation) and competitive assay performance (sensitivity, specificity) in comparison with in-house gold standard indirect immune fluorescence testing (IIFT) and commercially available test kits. Particularly, the newly developed CCHFV IgG IC ELISA was superior to the commercial IgG ELISA in detecting the rising IgG titers during the acute phase of the disease. Due to the proven cross-species reactivity of the recombinant capture molecules used for ELISA plate coating, sera originating from livestock, e.g. cattle can be analyzed using the same set of reagents as for the human samples. The CCHFV being a zoonotic pathogen transmitted to humans either by tick bite or by contact with tissues or blood of an infected animal, this veterinary application is also highly relevant for risk assessment of human infections.
NP 13

Need for biosafety and biosecurity networking structures in Sudan

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Background: The Sudanese National Biosafety and Biosecurity Policy (NBBP) was developed and endorsed in 2017 by the Sudanese Federal Ministry of Health (FMoH) with support of the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH under the framework of the German Biosecurity Programme. It created the legal basis for improvements in the field of biosafety and biosecurity with one focus on networking structures. This study examines the current need to further extend the National Laboratory Network on Biosafety and Biosecurity (NLNBB), founded in 2016 by the FMoH with support by GIZ. The NLNBB’s task is to facilitate and coordinate all measures to improve biosafety and biosecurity in Sudan.

Methods: A series of meetings and site visits, from March 2017 to June 2018, were done to analyse the need and obtain consensus from laboratories across Sudan to join the NLNBB. This survey therefore was executed as part of an enrolment process in the network. Information regarding knowledge, attitude and skills of laboratory biosafety and biosecurity, availability and proper use of personal protective equipment (PPE), attitude on and use of standard laboratory practices, and biosafety / biosecurity awareness was obtained. The data was analysed with descriptive statistics to construct a roadmap for the further development of a fully functional laboratory network.

Results: A total of 21 laboratories from 14 Sudanese states participated in the study. The result of the survey revealed the following: 46.6% of respondents received a low score (scores < mean +1 standard deviation) regarding a general knowledge about biosafety and biosecurity. Very few (10%) reported availability or use of PPE. 35% of the participants reported no access to biosafety level BSL 2 - 3 facilities. None reported prior experience with BSL - 4 laboratories. Knowledge scores pertaining to biosafety and biosecurity management practices were 47.3%. Only 40% of respondents (from 8 laboratories) reported having biosafety / biosecurity officers. The majority (65%) were unaware of laws and regulations guiding biosafety and biosecurity.

Conclusion: The current limited knowledge, attitude and practice of laboratory biosafety and biosecurity among the participating laboratories necessitates the need for the extension of biosafety and biosecurity networking structures, to facilitate building a culture of safe and good practices of biomedical laboratory work according to international standards in Sudan.

NP 14

The role of Germany Biosecurity Programme in improving laboratory biosafety and biosecurity in Sudan

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Sudan is located in the heart of Africa continent which has different geographical areas. Sudan shares borders with seven countries, of which some are suffering from conflict, starvation and droughts resulting in a considerable influx of migrants into Sudan. No doubt, laboratories are the first line of defense in the Sudanese Ministry of Health because they are dealing with a wide range of pathogens, including previously unknown and emerging pathogens, often due to changing situations and migration.

In addition, Sudan is an area at great risk of communicable diseases outbreaks, in both, human and animal populations. Therefore there is a need for biosecurity measures in order to avoid spread of disease either through naturally occurring outbreaks, accidents or through the deliberate misuse of biological agents. The large variety of biological agents investigated within the laboratories demands a reliable protection of laboratory personnel and laboratory staff capacity to combat these threats. Sudan is a key partner in the German Biosecurity Programme. The programme was launched within the engagement of the German Federal Foreign Office as a contribution to the G7 “Global Partnership against the Spread of Weapons of Mass Destruction” in 2013. Currently, the programme is in the second funding period (2016-2019).

The aims of the German Biosecurity Programme are to:

- Improve mitigation of biological risks
- Enhance Sudanese public health capacities
- Foster international scientific exchange and quality scientific personnel
- Contribute to the fight against dangerous diseases
- Support safe and secure working conditions for handling dangerous pathogens and toxins
- Secure and account for materials that represent biological proliferation risks

The implementation of the programme uses a uniform methodological approach which focuses on
capacity development in five major components: Detection and Diagnosis, Biosafety and Biosecurity, Surveillance, Awareness raising and Networking. This presentation will explain the role and importance of the German Biosecurity Programme to the National Public health laboratory (NPHL), which functions as the central reference laboratory within the Sudanese Federal Ministry of Health.

**NP 15**

Public Health Risk Communication Strategy and Public Health Risk and Response Communication Master Plan (Schéma Directeur)

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Moroccan Ministry of Health has since the 2009 pandemic AH1N1 flu initiated the implementation of communication strategies and plans. The implementation of communication activities is under the responsibility of the Ministry of Health, which coordinates its interventions with ministries and government departments. In addition, a National Action Plan for Sanitary Security was drawn up in 2015. One of the main recommendations from the national plan is to create mechanisms for coordination of public health risks and public health emergency communication between actors and stakeholders from different sectors. The implementation of communication activities during different public health events, in national or international scope, has often revealed the need to harmonize and validate multisector activities and corpora of messages, communication actions, mass media and institutional communication interventions. For that, the German bio-safety and biosafety program has supported the Ministry of Health during 2015 - 2018 to develop a complementary framework for public health epidemiological and emergency response plans: The health and safety risk communication strategy and the master plan for risk communications and public health emergencies. The purpose of this device is: to contribute to better managing public health risks, to be able to communicate transparently in crises; To Promote informed decision-making, positive behaviour change, and the maintenance of public trust and community mobilization.

**The health risk communication strategy** based on three axes: Information, Coordination and Communication. The Coordination axis provides for the establishment of a coordination mechanism between the different national sectors and the various stakeholders involved in risk communication in public health. A master plan for risk communications and public health emergencies has been proposed.

**The master plan for risk communications and public health emergencies** describes coordination of intra-sectoral and intersectoral intervention in the area of communication. It’s built on ethical principles and rules concerning the exchange and coordination of information and communication activities. A National Communication Group on Public Health Risks and Emergencies is expected to animate and coordinate communication on risks and emergencies between different sectors.

**NP 16**

First molecular biological analysis of tick-borne encephalitis virus in Akmola Oblast and East Kazakhstan Oblast

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Tick-borne encephalitis (TBE) is endemic in Eurasia and is one of the most dangerous neuroviral infections in humans. In Kazakhstan (KZ), 216 TBE infections were reported from 2011-2016. We focused on two regions of KZ, the endemic region Eastern Kazakhstan Oblast (EKO) and a region not endemic for TBE, Akmola Oblast (AO). Our goal was to determine the minimum infection rate (MIR) of TBEV in ticks, to assign the TBEV subtype and to perform a first phylogenetic analysis.

In 2016, 1522 ticks from 26 districts, in 2017, 1063 ticks from 27 districts were collected in EKO and 437 ticks from one district in AO..Ticks were sorted into 455, 333 and 90 pools, respectively. After homogenization, tick pools were tested by TBEV real-time RT-PCR. Subsequently a E-gene RT-PCR, sequencing and molecular analyses were carried out. TBEV RNA was detected in 5 pools of EKO (MIR 0.5%) and also in 2 pools of AO (MIR 0.5%). TBEV was found in *Ixodes persulcatus* and in AO also in *Dermacentor marginatus*. TBEV from both oblasts belong to the Siberian subtype. Phylogenetic analysis showed that the new TBEV strains from EKO are closely related to strains from China and Western Siberia. TBEV from AO are related to strains from Western Siberia.; In this study, we show first
molecular biological data on TBEV in a previously known endemic region in EKO. In AO, which was not endemic, we detected TBEV for the first time in ticks. Our data will directly contribute to improving the surveillance and prophylactic countermeasures in the two regions in KZ.

NP 17
Prevalence of Rickettsia species in ticks in Kazakhstan

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Over 60 years ago clinical patterns resembling tick-borne rickettsioses have been described for the first time in Kazakhstan. Since 1995 the incidence of clinical cases in humans seems to be rising but studies on epidemiological data regarding the occurring etiological agent, tick vector species, prevalence, and distribution throughout Kazakhstan are still scarce to date. In order to identify Rickettsia (R.) species occurring in Kazakhstan, we collected and investigated Ixodes (I.) persulcatus (n=1193), Dermacentor (D.) niveus (n=523), Haemaphysalis (Ha.) punctata (n=470), Hyalomma (Hy) asiaticum (n=77), Dermacentor (D.) marginatus (n=55), Dermacentor (D.) reticulatus, Rhizophagus (Rh.) turanicus (n=9) for the presence of rickettsial DNA from three districts each of the Almaty and Kyzylorda regions. The calculated Rickettsia Minimum Infection Rate (MIR) in the investigated ticks in Almaty region varied between 0.4% and 15.1% and between 12.6% and 22.7% in the Kyzylorda region. At least five different Rickettsia species were identified during our research in the two selected regions of Kazakhstan. Two of them are already known to science: R. raoultii and R. slovaca, the latter being for the first time described in this region. One new Candidatus R. yenbekshikazakhensis and one new Genotype R. talgarensis were detected in Almaty region. The role of these Rickettsiae for human diseases has further to be investigated.

NP 18
Seroepidemiological and molecular investigations of subclinical infections with Crimean Congo hemorrhagic fever virus in Kazakhstan

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Crimean-Congo hemorrhagic fever (CCHF) is endemic in South Kazakhstan. To detect subclinical cases of CCHF-virus (CCHFV) infection, paired serum samples from 802 patients with fever of unknown origin (FUO) were investigated in two oblasts: Kyzylorda oblast (region) is known to be endemic CCHFV, Almaty is so far a none-endemic region for CCHF. In 12.7% of the sera IgG antibodies were detected indicating that subclinical CCHFV infections are present in both oblasts in Kazakhstan. Thus, for the first time these results gave evidence for the circulation of CCHFV in Almaty oblast. Acute infection was shown by IgM-ELISA in four patients from Kyzylorda, with only one developing a severe hemorrhagic form of CCHF. In three patients’ sera viral RNA was detected by RT-PCR. Phylogenetic analysis revealed CCHFV genotype Asia 2 and a possible reassortment between the genotypes Asia 1/ Asia 2. Animal husbandry such as cattle and horses were significantly associated with CCHFV seropositive patients. In conclusion, subclinical CCHF is present in Kazakhstan. Therefore, physicians treating patients with FUO should be aware of this.
The epidemiological influence of tick-borne encephalitis in the southern part of Kazakhstan

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Background and objectives: One of the most severe arboviral infections in Kazakhstan is tick-borne encephalitis (TBE). TBE is a serious health problem in Kazakhstan with up to 50 cases per year. Therefore, we examined the prevalence of antibodies against TBE virus (TBEV) in humans suffering on fever of unknown origin (FUO) and the presence of TBEV in ticks from six districts in Almaty (AO) and Kyzylorda oblast (KO).

Materials and methods: In the six districts of AO and KO 2341 ticks were collected. Ticks were sorted in pools and homogenized. Extracted RNA was screened for the presence of TBEV. Products of a conventional E-Gen RT-PCR were sequenced and a phylogenetic analysis was carried out. Paired sera (day 0 and 10-14 after hospitalization) from 795 patients with FUO from 13 hospitals in AO and KO were investigated for the presence of IgG/IgM against TBEV.

Results: The Minimum Infection Rate (MIR) of TBEV in three districts of AO are between 1.1% and 4.4% whereas in the districts of KO none of the tested pools were TBEV positive. Sequencing results show that the TBEV belongs to the Siberian subtype. TBEV IgG antibodies were detected in 23 (2.9%) out of 795 sera samples.

Conclusion: In this study, we present new data on the circulating TBEV subtype in ticks in AO. For the first time, a serological study was carried out to rule out the role of TBEV as a cause of FUO in Kazakhstan. Our results will help to improve TBEV surveillance and prevention of this infection in Kazakhstan.

FLI – LANAVET Collaborative research on zoonotic viral hemorrhagic fevers in Cameroon

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The research collaboration between FLI (in Germany), LANAVET (in Cameroon) and other African partners on zoonotic viral hemorrhagic fevers started on the project: «Presence and prevalence of Crimean Congo Hemorrhagic Fever (CCHF), Rift Valley Fever (RVF) and Nipah(-like) viruses (NiV) in Mauritania, Sierra Leone, Cameroon and the Democratic Republic of the Congo ». The objective and the overall strategy of this collaborative project is capacity building in African partner countries to detect the presence these cited pathogens by modern molecular and serological techniques. In Cameroon, samples (sera, swaps) of various animal species namely bovine/ovine/caprine (3000), equine (124), canine (25), porcine (316), and the Eidolon helvum bats (284) were collected across several regions of Cameroon and screen using developed in-house qPCR and ELISA. Furthermore, samples of mosquitoes (over 12,000) and ticks (over 20,000), potential vectors were also collected. Some training courses were organized at LANAVET and some LANAVET staff visited FLI for laboratory training on the detection of these pathogens. This project allows LANAVET to effective gain in capacity building and technology transfer. Our findings show that the PCR detection of CCHFV was present in Cameroon by qPCR and ELISA for the first time on the detection of these pathogens. This project allows LANAVET to effective gain in capacity building and technology transfer. Our findings show that the PCR detection of CCHFV was present in Cameroon by qPCR and ELISA for the first time with a seroprevalence of 74%. More importantly, CCHFV-RNA was detected in 7 Hyalomma ticks out of 109. In addition, acute case of RVFV was confirmed by IgM in-house ELISA and PCR with specific antibodies in cattle (13.5%) and small ruminants (3.4%) from 2013-2014 samples. Entomological studies have highlighted the presence of several species of mosquitoes and ticks. This collaboration was very beneficial for both partners with more gain to LANAVET, the Cameroon government as well as the scientific society worldwide.
NP 21
How the German-Kazakh network for biosafety and biosecurity supports biorisk management in Kazakhstan

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Due to the various environmental conditions Kazakhstan offers a wide range of natural foci for epidemiologically significant diseases like plaque, tularemia, tick-borne encephalitis and Crimean-Congo hemorrhagic fever. All are potential pathogenic B-agents and can be a threat to the public health, be it by appearing accidentally in the laboratory, by being intentionally misused or in a terrorist attack. One aim of the German Biosecurity Programme is to identify biorisks while handling relevant pathogens in biological laboratories (BL). To reduce the possibility of any misuse or accidental release of highly dangerous pathogens in BL’s a good Biorisk Management System is necessary. Herein we report an example how the WHO Biorisk Management System can be adapted by our cooperation partners in Kazakhstan and how it will help to increase biosafety and minimize the potential risk of human infection by dangerous pathogens.

In our Risk Assessment we identified several risks in the BL; i.e. crushing potentially infected ticks without carrying personal protective equipment (PPE) and without using a biosafety cabinet (BSC). As Mitigation Measures the laboratory was equipped with new furniture, a BSC and a tissue lyser that allows homogenization of ticks free of aerosol. Standard Operating Procedures were implemented for all necessary procedures to be carried out in the BL including PPE. To minimize the risk of laboratory infections we started to educate the laboratory staff in several tabletop exercises to raise their awareness. Effective Performance was achieved by setting up a laboratory quality system in which proper procedures are embedded. Briefings and internal audits are given to the laboratory on a regular basis.

The establishment of a Biorisk Management System for our cooperation partners has proven to effectively increase the risk awareness in the local BL’s. The laboratory setting is a good example of how Biorisk Management becomes a necessity in those environments and how easily biosafety and biosecurity can be carried out once a good management plan is in place.

NP 22
Capacity building for the biosecure diagnostics of Rift Valley fever virus and Crimean-Congo hemorrhagic fever virus infections in animals in Mauritania

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Rift Valley Fever virus (RVFV) is an arthropod-borne Phlebovirus (Bunyaviridae family) affecting humans and a wide range of vertebrate hosts like sheep, goats, cattle and camels. The virus causes large outbreaks with high neonatal mortality in livestock, abortion in pregnant animals and haemorrhagic fever in humans. Crimean-Congo hemorrhagic fever virus (CCHFV) is a widespread arthropod-borne virus which is distributed in many parts of Africa, Asia and Europe. It is mainly transmitted by ticks of the genus Hyalomma. CCHFV can cause severe hemorrhagic fever in humans with case fatality rates of 5 - 30% or higher. However, infected ruminants and camelids do not show any signs of clinical symptoms, which makes it very difficult to narrow down specific risk areas.

Within the framework of the German Biosecurity Programme (“Minimizing Public Health and Bioterror Risks by CCHF and RVF Viruses in Mauritania, Cameroon and Sierra Leone”) the prevalence and distribution data will be determined for Rift-Valley fever virus (RVFV) and Crimean-Congo hemorrhagic fever virus (CCHFV) in Mauritanian livestock (cattle, camels and small ruminants). One main objective of the project is the capacity building in the African partner countries to determine hemorrhagic fever viruses (e.g. CCHFV, RVFV) by modern molecular and serological techniques. In a collaboration of ONARDEL and FLI, mosquitos and ticks were sampled in different regions in Mauritania and analyzed to assess the true distribution of RVFV and CCHFV. To ensure independent diagnostics of samples in case of possible outbreaks, scientists of the ONARDEL were trained regularly in workshops by FLI specialists in molecular and serological diagnostic methods. After establishment of the methods ONARDEL should be able to perform the surveillance activities autonomously in Mauritania. Based on obtained data, risk areas will be defined and public protection methods as well as pathogen ascertainment procedures will be implemented.
NP 23
Crimean-Congo hemorrhagic fever and African swine fever virus infections in animals in Ukraine

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Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus, belonging to the genus Orthohantavirus in the family Hantaviridae. It was first described as a discrete human disease in the Crimean region of the former Soviet Union in 1944. CCHFV can cause a severe hemorrhagic fever in humans with case-fatality rates of up to 30%. Inside Europe, its distribution closely correlates with the distribution of Hyalomma ticks, which are both vector and reservoir of CCHFV.

African Swine Fever (ASF) is a highly significant haemorrhagic viral disease that affects only porcine species (both wild and domestic) of all breeds and ages, giving rise to a variety of clinical signs and lesions. The disease was first described in 1921 in Kenya and is endemic in several African countries. In 2007, the disease was introduced into Georgia and spread since then over the Trans-Caucasian countries and Russia into Belarus, Ukraine, Moldova and European Union Member States Estonia, Latvia, Lithuania, Poland, Czech Republic, Hungary, and Romania.

Within the German Biosecurity Programme, activities were undertaken to monitor these diseases in Ukraine, by introducing, verifying and where necessary improving laboratory biosafety standards, novel animal disease diagnostics, and giving also methodological support for regional investigation offices. Scientists from SSRILDVSE took trainings at FLI and in their home institute on diagnostic molecular genetics and serological methods for CCHF and ASF. Workshops on tick vector collection and on the specific definition were given, in order to determine the tick distributions. Altogether 1500 ticks and 2020 serum samples (from cattle and goats) were obtained and analyzed. Present CCHFV infection rates in ruminants and ticks will be presented. For a better understanding of the ASF epidemiology, next-generation sequencing approaches have been optimized using a set of recent Ukrainian ASFV strains, and enrichment protocols for viral genomes were established. The yield of viral sequences allows now for in-detail molecular epidemiological studies in Ukraine and integration into broader studies of all affected countries.

NP 24
Seroprevalence and risk factors of Rift Valley fever in small ruminants in Northern Cameroon

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A cross-sectional study was conducted from April 2016 to June 2017 on seroprevalence and risk factors of Rift Valley Fever in domestic small ruminants in the North Cameroon region. Sera were collected from 680 small ruminants randomly selected (355 goats and 325 sheep) in 8 localities and 3 departments. The competitive ELISA was used to determine antibodies against the Rift Valley Fever virus. The results obtained showed an overall seroprevalence of 3.4% (95% CI: 2.0 - 4.7%). Sheep were the most affected with a prevalence of 4.6% (95% CI: 2.3 - 6.9%) versus 2.3% (95% CI: 0.7 - 3.8%) in goats. Bénoué division was the most affected with a prevalence of 5.1% (95% CI: 3.0 - 7.2%) followed by Mayo-Rey (0.8%; 95% CI: -0.8 - 2.3%). The highest prevalences were recorded in the localities crossed by the Bénoué River (Lagdo, Pitoa). Age (OR = 6.3, 2.2 - 18.9), sex (OR = 4.2; 1.0 - 18.1), access to water points (OR = 6.3, 2.3-17.3), season (OR = 4.7, 1.6-13.9), and locality (OR = 14.3, 1.4-143.1) had a significant influence on the presence of RVF antiviral antibodies, and would then be the main risk factors for the emergence of the disease. Considering these serological results, the Rift Valley Fever virus could be circulating in the North Region of Cameroon. A surveillance program for this disease should be set up by the Cameroonian government and could take into account the regulation of cross-border movement of...

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Two types of traps were used for identification of mosquito species in the districts of southern Azerbaijan and for conducting the required preliminary ecological studies. The areas with the greatest likelihood of presence of bloodsucking mosquitoes, as well as those in the vicinity of water sources and forests with versatile landscapes were the main criteria for selecting the villages. The purpose of the study is to collect flies from these ecological environments using two types of traps, which will give you an idea of which of these traps is more effective for the research, as well as find out about the species and distribution of mosquitoes in the study area.

The mosquitoes were collected as part of CBEP projects in Azerbaijan during 2015 – 2017 using CO2-baited 8 CDC light traps and 2 BG-Sentinel traps using a chemical lure (BG-Lure) which mimics the body odor of the host organisms. The mosquitoes were stored on ice until transportation to the laboratory and killed by freezing for subsequent identification and pooling.

14310 mosquitoes with CDC light traps and 1971 mosquitoes with BG- Sentinel traps from 5 collection periods were collected during the study. 5422 mosquitoes were identified and as a result 17 mosquito species from 6 genera were detected. The dominant species of mosquitoes Culex pipiens (36.6%) and Aedes vexans (33%) were found in both traps, but less common Culex ministicus (9%), Mansonia richardii (0.17%) and Culiseta longiareolata (0.05%) were caught only in the BG-sentinel traps, which proved the effectiveness of this type of traps. The remaining unspecified mosquito samples are stored in RAPS -80°C freezer. These samples were not processed and can be used for future studies.

The information to be obtained is important for the development of targeted public health measures. It will also help decide which type of the trap is more effective, what species of mosquitoes it can help catch, and which type of virus (the West Nile virus and the Sindbis virus) these mosquitoes transmit.
Enhancing biosafety and biosecurity in Ethiopia for control of health threats

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Control of health threats is challenged by several factors; among which rapid growing human population particularly in developing countries and globalization are the major ones. Competence, information and technology are becoming most important commodities in controlling global health challenges. Training health professionals from different disciplines is of paramount importance in reducing health threats and enhancing Biosafety and Biosecurity. Moreover, initiation of Biosafety and Biosecurity associations composed of professionals from wider disciplines play a paramount role in addressing health challenges in an integrated and coordinated approach. Though Ethiopia has trained professionals in health, and biosafety and biosecurity, there is no any biosafety and biosecurity association in the country. Therefore, there is a need to initiate a Biosafety and Biosecurity association in the country to better contribute for the control of health threats. Considering its importance, we are in the process of initiating Ethiopian Biosafety and Biosecurity association. At the moment, a group of 6 International Federation of Biosafety Association (IFBA) certified professionals (3 from Mekelle University, 2 from Armeur Hansen Research Institute and 1 from Ethiopian Public Health Institute) and one expert from Ethiopian Ministry of Health are in the process of initiating the Ethiopian Biosafety and Biosecurity Association. Two GIBACHT trained professionals from Ethiopia are also invited to join the team. The Global Initiated Biosecurity Academia for the Control of Health Threats (GIBACHT) program has helped me through providing practical oriented training on Health issues, Outbreak Investigation and Surveillance, Biosafety and Biosecurity, Integrated Disease Control, etc. The training that I got from GIBACHT has also helped me in getting the IFBA.

“SHOCRoom” - a Tunisian model of Public Health Emergency Operations Centre

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Regardless of their origin, emergencies and disasters require capacities to collect and analyse real-time information, to assess the public health impact, and to trigger an on-time and coordinated response. Public Health Emergency Operation Centres (PHEOCs) are a core component of a country’s Public Health system which helps ensuring those capacities and offering a platform for optimal response operations. Our aim was to describe and evaluate the Tunisian PHEOC according to the WHO Framework for a PHEOC issued in November 2015.

Methodology: The PHEOC was evaluated using the checklists of Annex 3 (systems and infrastructure requirements) and Annex 9 (implementing a PHEOC)

Results: In February 2010, the Tunisian Ministry of Health established its national Strategic Health Operations Centre (Shocroom) with support from WHO. It is a permanent structure with 9 staff, a dedicated workspace and two assigned key functions: Emergency Preparedness and Response and offering a communication interface with the regions. In absence of public health threats, information from public health structures and extrasectoral partners is continuously monitored 24/7. Potential public health threats can be notified to the Shocroom through a dedicated communication interface including telephone, email, fax and videoconferencing by the public, physicians, paramedics, administrative staff and other sectors, like civil protection and the military. In case of potentially major events, Shocroom coordinators liaise with experts in the concerned field to decide whether the alert situation Incident Management System (IMS) needs to be activated. An IMS was activated in response to the mass gathering at the Tunisian-Libyan border in 2011 and following the terrorist attacks in 2015. The Shocroom fulfils basic EOC coordinating functions as well as public health-related functions like situation monitoring and risk assessment. However, the organizational structure is neither easily scalable nor flexible, as it is managed by emergency department staff of in and outside of crisis situations. Surge staff is engaged for some emergencies. The Shocroom has a clear information management framework including information collection, assessment and dissemination.

Conclusions: The Shocroom’s capacities as a permanent PHEOC can be described using the WHO framework. However, a more in-depth evaluation could help making targeted recommendations and setting up an action plan to improve its performance.
**NP 29**
Sectoral reorganization of Public Health emergency management in Morocco, 2018

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**Introduction:** Threats and risks in public health have become recurrent. They can be biological, chemical, radiological or nuclear, natural, accidental or intentional. In Morocco, the Ministry of Health (MoH) has been called upon to manage several public health emergencies (PHE): SARS, Pandemic AH1N1, anthrax, natural disasters etc. The coordination of these emergencies within MoH is provided by the Department of Epidemiology and Disease Control (DELM) with the involvement of the Central Coordination Center when necessary. At the regional and provincial level, the risk and PHE management is coordinated by the public health services and the provincial epidemiology units. Despite this well-structured organization, certain gaps have been identified: dissociated surveillance and response, duplication of tasks and partly long delays concerning data transmission.

**Objective:** Reorganization of the sectoral management of public health emergencies and implementation of Public Health Emergency Operations Centers (PHEOC) in order to ensure early detection and response in case of PHE and threats, as well as to control epidemics and reduce their impacts.

**Method:** Organization of a workshop led by World Health Organization (WHO) experts with the support of Deutsche Gesellschaftfür Internationale Zusammenarbeit (GIZ) in September 2017, also including group work. Benchmarking during the annual meeting of the Emergency Operations Center (EOC)-network, and training of rapid response teams (RRT).

**Results:** Through the collaboration of WHO and the Eastern Mediterranean Public Health Network (EMPHNET), 135 people were trained in RRT with the establishment of two central RRT and 18 provincial RRT. 19 regional focal points have been trained. In addition, the support of the German Biosecurity Programme has allowed developing a roadmap to set up a national PHEOC. The main organizational and functional lines of the national and regional PHEOC have been drafted and discussed within DELM, aiming to create one National and 12 Regional PHEOC. A health ministry decision to institutionalize this reform is being prepared.

**Conclusion:** This reform, which follows a coherent process based on best practice and international standards, must be realized quickly by setting up structures and teams with the development of organizational and functional procedures. This effort is supported by the German Biosecurity Programme.

**NP 30**
Kazakhstan’s experience of BS&S specialists training in the framework of EU CBRN CoE Project 53: Strengthening the national legal framework and provision of specialized training on biosafety and biosecurity in Central Asian countries

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As part of the Project 53 of the European Union Chemical Biological Radiological and Nuclear Risk Mitigation Centres of Excellence Initiative (EU CBRN CoE) (www-coe project 53.istc.int) Kazakhstan conducted an institutional and laboratory education needs assessment in 2017. Based on this assessment a sustainable Biosafety and Biosecurity (BS&S) training program was developed. Initially 14 Kazakh experts were trained to be trainers by a European team of experts in Biosafety (Public Health England) and Biosecurity (RIVM) with support from Kazakh experts. Kazakh trainees were from 7 Universities (4 medical, 2 biological, 1 agricultural/veterinary), 4 National centres (2 medical, 1 biological, 1 veterinary) and Uralsk Anti Plague station to maximize the range of institutes that the new trainers could reach. Currently, six BS&S centers have been established in Almaty, Astana, Karaganda, Semipalatinsk, Shymkent and Uralsk. Additionally, BS&S course were successfully developed for their inclusion to the compulsory curriculum of medical, veterinary and biological students of the next (2018-2019) academic year.

15 BS&S workshops are planned in the framework of the Project 53 in 2018, in cooperation with EU specialists and using the newly trained trainers. To date trainings were provided to: 62 students in BS&S at Kazakh National Medical University, Almaty; 16 laboratory specialists at National Center of Expertise, Astana; 30 Extremely Dangerous
Pathogens (EDP) specialist at Uralsk Anti Plague station, Uralsk; 22 infectious diseases specialists at the Shymkent Regional Infectious Hospital. Future trainings will consists: Laboratory BS&S clinical, biochemical, serological, molecular, sequencing also bacteriological, virology, including EDP’s laboratory; Clinical (Human) BS&S, both pre-hospitals and hospitals, including infectious and EDP; hospitals; Field BS&S on sample collection of insects, arthropods, animals in natural and epidemi foci; veterinary BS&S in farm work; BS&S on custom work, on tranport, at emergency situations of natural or man-made character; BS&S related to terrorist threats, border and military. Here we present methodologies and strategies used in BS&S Training that has been delivered to date.

NP 31
The performance of biorisk management system at National Public Health Laboratory NPHL

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The Performance of biorisk management system at National Public Health Laboratory NPHL Sanaa Mirghani Babiker Hassan , Safia Gaafar Mohamed Ahmed National Public Health Laboratory, Ministry of Health, Khartoum Sudan National public health laboratory is key public health laboratory of the republic of Sudan consist of diagnostic laboratories dealing with investigation of variety of highly pathogenic agent and regulatory laboratories, therefore need for reliable protection from biological agent and toxins to protect working area and environment. The implementation of biorisk management system basis on gap analysis to identify gaps depend on International health regulation assessment (IHR LAT Assessment Annex 2) and other assessment conducted at NPHL (KAP study, ISO 15190 - 2012 checklist resulted in developing strategic plan for biorisk management to provide safe and secure condition for working with dangerous pathogen and toxin. A biorisk management system (BRM system) on the basis of the CEN Workshop Agreement "Laboratory Biorisk Management “(CWA 15793:2011) is currently being established by collaboration between Robert Koch Institute and biorisk department at National Public Health Laboratory (NPHL), Khartoum, Sudan within the framework of the “Sudanese-German partnership program for excellence in biological and health security”. The aims of implementation of BRM at NPHL are: 1- foster a safe & secure working environment 2- Identification of hazard and assessment of risk 3- Implementation the required mitigation measures needed for dealing with identified hazards 4- sets requirements that are necessary to control risks associated with the handling or storage and disposal of biological agents and toxins in laboratories and facilities. 5- monitoring and evaluation of effectiveness of mitigations measures CWA15793 gap analysis checklist for implementation of CWA 15793:2011 used to identify gaps conducted in march 2017. In order to allow for a gradual implementation of the BRM system at NPHL and considering the current situation and needs . Effective implementation of biosafety and biosecurity measures can contribute not only to minimizing the risks posed by natural, accidental and deliberate disease but also to minimizing the potential for the misuse of information.

NP 32
Implementation of the Epidemiological Telephone Conference (EpiTec) in Tunisia, 2016-2018

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Indicator-based surveillance is no longer sufficient to respond in a quick, flexible, and adequate manner to public health challenges. Therefore, event-based surveillance (EBS) becomes necessary to respond to urgent public health threats. In Tunisia, the weekly epidemiological teleconference (EpiTec), which is an innovative surveillance method providing a structured scientific platform for regular exchange about current events related to infectious diseases, was implemented in March 2016 in collaboration with Robert Koch Institute (RKI). Our aim is to share the Tunisian EpiTec experience, to cite the most frequently reported events and to assess challenges for sustainability.; Methods: Surveillance experts from the Strategic Health Operations Center (Shoc room), National Observatory for New and Emerging Diseases (ONMNE), Primary Healthcare Direction (DSSB), Pasteur Institute of Tunis (IPT) and focal points from 24 governorates jointly developed a standardized operating procedure (SOP) for EpiTec implementation during workshops from 2015 to

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In April 2016, a simulation exercise was conducted to refine the procedures and to share a real operational EpiTec experience. Participant satisfaction was evaluated using an online survey in August 2017 and the EpiTec SOP was adapted accordingly during workshops in September and October 2017. Findings: The first EpiTec was held on March 13, 2016. Since then, the median number of participants per conference was 10 ± 0.73 (range 4-16). The highest participation rate per governorate was 86.4%, followed by 77.3%. Out of 24 regions, 22 participated at least once and the average number of events reported per EpiTec was 3.5 ± 0.45 (range 1-7). The main reported events were hepatitis A, SARI/ILI, human rabies, tuberculosis and brucellosis. The evaluation in 2017 showed that the majority of the participants felt well prepared to actively participate in the teleconferences and that they found the required amount of time adequate. The continued participation of all stakeholders and an agreement regarding the kind of events that should be reported via EpiTec was recommended; Conclusion: EpiTec is a new surveillance method in Tunisia, commitment of all stakeholders and implementation of the recommendations from the evaluation are required to improve it. A training curriculum targeted at local, regional and national level is currently being developed to reinforce its implementation.

**PP 01**

**Epidemiology of human foodborne botulism in Azerbaijan**

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Botulism is an emerging, serious disease caused by a neurotoxin produced from the spore-forming bacterium *Clostridium botulinum*. The toxin is the most potent biological toxin. Botulism in humans is caused by toxin types A, B, E. There are three types of botulism: food, wound, infant botulism. Foodborne botulism, the most common form, is caused by eating food containing preformed botulinum toxin and can cause large outbreaks. Cases of foodborne botulism are reported annually in Azerbaijan. The aim of this study was to identify the epidemiology of human foodborne botulism in Azerbaijan. Data set of all botulism cases from 2012 to 2016 was obtained from the Electronic Integrated Disease Surveillance System (EIDSS). Demographic information and epidemiological links were analysed using the Analysis Visualization Report module of EIDSS and Epi Info 7.0. The diagnosis of botulism based on clinical findings, history of exposure to suspect foods and laboratory diagnostics. The laboratory diagnostics is associated on the detection of toxin in the patient. The toxin detection is confirmed on the mouse lethality assay. The detection of *C. botulinum* in patient samples, such as feces, gastric and intestinal contents supports the diagnosis. During the 5-year period, a total of 228 confirmed botulism cases were reported. The number of cases increased by 13% in 2013 compared to 2012. In 2014, the number of cases decreased by 7%. The number of cases increased 10% in 2016 compared to 2015. The majority of cases occurred during winter months. Botulism occurs in different age groups, most cases are registered in the 30-59 year age group. Of all reported cases females were dominated by 56%. The most frequent source is home canned vegetables 85%. Other risk factors include sausages - 8%, smoked fish - 7%. For 5 years the number of cases has increased. The use of home-canned vegetables has been identified as a potential source of botulism. Control measures included to carry out sanitary-education work among the population about the danger of botulism when conserving food products at home.

**PP 02**

**High Phlebovirus seroprevalence in Austrian Army personnel returning from missions abroad**

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Phleboviruses belong to the prominent group of Arthropod-borne (Arbo) viruses and are transmitted by either sandflies, mosquitoes or ticks to several vertebrate species, including humans. Infections are often asymptomatic, but can result in a self-limiting febrile illness with sudden high fever, headache, photophobia, malaise and retro-orbital pain, or also severe diseases such as meningitis and meningoencephalitis. To date, no vaccine or chemoprophylaxis against phleboviruses are available, however a lifelong immunity to the respective serotype is established after a Phlebovirus infection. In sandfly-endemic regions, seroprevalences in humans as well as domestic animals are known to be high. Military personnel are at an increased risk for Phlebovirus infections, particularly when large numbers of immunologically naïve soldiers are introduced to Phlebovirus-endemic regions. High sandfly biting rates and considerable numbers of Phlebovirus infections have been reported from military troops operating in endemic areas. However, data on Phlebovirus infections in European Military personnel are still scarce. The aim of this study was to assess seroprevalences in Austrian soldiers returning from missions abroad and to evaluate potential risk factors associated.

A retrospective serological study was performed with 753 healthy Austrian soldiers returning from missions in Bosnia and Herzegovina (BIH), the Kosovo, Syria and the Lebanon and of soldiers prior to their missions. An indirect-immunofluorescence assay with subsequent fluorescence microscopy was used to detect anti-Phlebovirus IgG antibodies. Altogether, 115 sera (15.27%; 115/753) were positive for anti-Phlebovirus IgG antibodies, with highest seroprevalences found in soldiers returning from the Kosovo (20.69%; 54/261), followed by Syria (17.82%; 18/101), the Lebanon (14.29%; 9/63) and BIH (11.48%; 7/61). Of the soldiers tested prior to their missions 11.61% (31/267) were positive. Phlebovirus seropositivity significantly correlated with the duration of the missions (OR=2.5; 95% CI=1.0-6.6, p-value=0.04), with the outdoor activity “running” during the missions (OR=2.31; 95% CI=1.2-4.7, p-value=0.007), and with Leishmania seropositivity (OR=2.1; 95% CI=1.0-4.25, p-value=0.04).

This study provides the first data on Phlebovirus seroprevalence in Austrian Army personnel and the study indicates that soldiers are at significant risk to be exposed to sandflies and to infection with phleboviruses during missions.
Free Livinig Amoebae as hosts for “Giruses” and vectors of microorganisms with “Public Health” significance

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Free living amoebae (FLA) pose a considerable risk regarding environmental health and public health significance. While FLA are known as parasites of both humans and animals causing a wide range of symptoms they can also act as vectors of for phylogenetically diverse microorganisms, called endocytobionts.

Among those digestion- and lysis-resistant microorganisms there are human pathogenic microbes evoking individual medical problems and on a broader scope Public Health concerns.

FLA often serve as vectors of those microorganisms from the environment to humans. The endocytobionts are transported and protected by the FLA, among them bacteria, viruses, protozoa or fungi. A considerable diversity within FLA was published recently representing a range of pathogenic waterborne, food borne and soil organisms.

The relationship between FLA and their endocytobionts has an influence on evolutionary processes, a fact, that could be demonstrated when detecting the so called “Giant viruses“ (= giruses). Mimiviruses and Pandoraviruses are examples for interesting viral endocytobionts within FLA.

The development of (human) pathogenicity and virulence is associated with FLA and intracellularly residing bacteria. Environmental and climatic changes (whether arising from nature or human influence) are affecting FLA abundance, which may lead to an increase of infectious diseases associated with FLA or their endocytobionts.

Factors associated with Rift Valley fever virus exposure in humans, South Africa, 2015-2016

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Introduction: Rift Valley Fever virus (RVFV) causes periodic outbreaks with disastrous effects on animal and human health. Human exposure risks are important to identify for optimizing infection prevention strategies.

Methods: We conducted a cross-sectional serological and questionnaire survey among farm workers and veterinary professionals working with livestock (cattle, sheep and goats) and game farms within a RVFV outbreak-prone area in the Free State and Northern Cape Provinces in South Africa during 2015-2016.

Background: In recent years, the incidence and fatality rates of tick-borne encephalitis (TBE) in Mongolia have been increasing with new cases being registered in areas, where without the main tick vector. Therefore, we aimed to determine the characteristic of species of Dermacentor tick for tick-borne encephalitis in the natural foci of Mongolia.
2015-2016. Sera were tested for immunoglobulin G antibodies using RVFV-recombinant antigen-based indirect and inhibition ELISAs. Factors potentially associated with RVFV seropositivity were assessed using unconditional logistic regression accounting for within-farm clustering.

**Results:** The RVFV seroprevalence was 8.9% (61/685) (95%CI:6.8-11.6%) among farm workers and 8.0% (11/138) (95%CI:4.6-13.5%) among veterinary professionals (p=0.869). There was also no difference in seroprevalence between workers on private compared to communal farms (p=0.654). On multivariable analysis factors associated with higher RVFV seroprevalence in farm workers were: (i) older age (number of large epidemics experienced) [40-63 years (2): adjusted odds ratio (aOR):5.0 (95%CI:2.3-10.8); ≥64 years (3): aOR:10.8 (95%CI:2.6-45.1) compared to <40 years of age (1)], (ii) injecting or collecting blood samples from animals [aOR:2.5 (95%CI:1.1-5.9)], (iii) slaughtering animals [aOR:4.9 (95%CI:1.2-19.4)], (iv) consuming hooved animals found dead [aOR:3.4 (95%CI:1.6-7.5)], and (v) working on farms where water was dammed [aOR:4.4 (95%CI:1.5-13.0)]. In veterinary professionals, older age (number of large epidemics experienced) was also associated with higher RVFV seroprevalence (p<0.01).

**Conclusion:** Improving precautions during injection, sample collection, slaughtering and meat consumption could lower the risk of RVFV infection.

**PP 07**
WITHDRAWN

**PP 08**
Serological evidence of typhus group and spotted fever group rickettsia exposure in humans, associated with molecular detection of *Rickettsia* spp. in fleas from Madagascar

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Rickettsiae are obligate intracellular bacteria, including 2 potential bioterrorism agents, responsible for many febrile syndromes and emerging infectious diseases ranging from middle to severe infections around the world. The current study was conducted in order to assess exposure of Malagasy population to spotted fever group (SFGR) and typhus group Rickettsiae (TGR), to determine the socio-economic and environmental drivers that influence their exposure to these pathogens, and to assess prevalence of Rickettsiae in rodents’ fleas.

A cross-sectional study was conducted in 28 sites including urban and rural area. For each area, about 30 persons were randomly investigated in general population. A standard questionnaire was administered to consent participant, followed by blood sample collection. In the same time, rodents were trapped in houses, in their immediate vicinity and in outdoor trap-lines. Fleas were collected from trapped rodents and stored in 95% ethanol. Two group-specific ELISA were used to look for anti-SFGR and anti-TGR IgG in human sera. To assess rickettsial exposition risk factors, serological status were used as response for statistical analyses at individual and site level with Generalized Linear Mixed Model. Fleas were subjected to DNA extraction, and then screened for *Rickettsia* sp. using genus-specific qPCR, followed by *Rickettsia typhi* and *Rickettsia felis*-specific qPCR.

Of 1669 included human participants, seroprevalence of anti-SFGR and anti-TGR IgG were 41.9% and 21.5%, respectively. Exposure risk to SFGR increases with age, is higher in men, in rural areas, and in areas with hot and non-seasonal climate; but decreases inversely with the education level, probably related to occupation or living standards. Exposure to TGR (more likely *R. typhi*) increases with age, and is higher in urban areas that have high abundance of *Rattus norvegicus*. The humidity is also an important climatic factor, and is probably associated with optimal conditions for development of the different life stages of the flea vector. Out of 1356 tested *Xenopsylla cheopis*, 7.2% and 4.6% were positive for *R. typhi* and *R. felis*, respectively. *R. felis* was also detected in 4% (n=149) of an endemic flea, *Synopsyllus fonquerniei*.

We found evidence of exposure to SFGR and TGR pathogens in general population, and detected *Rickettsia* spp. in peridomestic rodents’ fleas, suggesting that Rickettsiae should be considered as causes of undifferentiated fever in Madagascar.

**PP 09**
Species and genetic diversity of tick-borne pathogens found in the area of sympathy of *Ixodes, Dermacentor*, and *Haemaphysalis Genera* Ticks in the Baikal region

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*IXODES*, *DERMACENTOR*, and *HAEMAPHYSALIS* Genera Ticks in the Baikal region
A comparative analysis of the species and genetic diversity of pathogens revealed in the sympatry zone of *Ixodes*, *Dermacentor*, and *Haemaphysalis* genera ticks. During the study of *I. persulcatus* ticks in this area the following pathogens were identified: tick-borne encephalitis virus of the Far Eastern, Siberian (‘Vasilchenko’ and ‘Zasaev’ lineages), European, Baikal (‘group 886’) subtypes, 178-79 strain; *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Babesia crassa*, *Rickettsia raoultii* (DnS28), *Candidatus R. tarasevichiae*. In *Haemaphysalis concinna* ticks DNA of *E. muris*, *A. phagocytophilum*, *B. crassa*, *B. microti*, *R. raoultii* (DnS28), *Candidatus R. tarasevichiae* were found. In the ticks of *Dermacentor* genus, only DNA of *Rickettsia sibirica* and *R. raoultii* (DnS14, DnS28) were detected.

A variety of tick-borne pathogens, detected in the organs of small mammals, living in the zone of sympatry of ticks’ three species. From the brain of *Myodes rutilus*, the strains of TBEV of the Far Eastern, European, Baikal genotypes were isolated; DNA of *A. phagocytophilum* was detected in the liver. In the organs of *Microtus oeconomus*, TBEV, *A. phagocytophilum*, *E. muris*, *B. microti* US-type were detected. TBEV strain of the European subtype was isolated from the brain of *Microtus gregalis*, and DNA of *E. muris* was found in liver. From the brain of *Spermophilus undulatus* gophers, TBEV strains of the European subtype have been isolated. In the samples of the liver of *Sorex araneus*, *E. muris* DNA was detected. DNA of *Anaplasma ovis* was detected in blood samples of domestic sheep. The study was funded by RFBR, research project № 16-04-01336-a.

### PP 10

**Gynandromorphism in Ixodes ricinus (Acari: Ixodidae)**

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Hyalomma marginatum, a two-host tick, is distributed in North Africa and southern Europe. It is known as an important vector of Crimean Congo hemorrhagic fever (CCHF) virus and *Rickettsia aeschlimannii*. In June 2018, an unusual female ixodid tick was found feeding on a sheep in Wächtersbach, in the Federal State of Hesse, Germany. The tick was identified morphologically and genetically as *H. marginatum*. Real-time PCRs were carried out to test for Crimean-Congo hemorrhagic fever (CCHF) virus and *Rickettsia spp.*. For the identification of *Rickettsia* species the 23S-5S intergenic spacer region PCR was used. The tick tested negative for CCHF virus. The screening for *Rickettsia* gave a positive result. The *Rickettsia* species was identified as *Rickettsia aeschlimannii*. This event shows that exotic tick species are imported into Germany, which carry human pathogens and therefore might be the source of exotic diseases acquired in Germany.
**PP 12**

**A model for forecasting the *Ixodes ricinus* activity**

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Ticks of the species *Ixodes ricinus* (L.) are the major vectors for tick-borne diseases in Europe. The aim of this study was to quantify the influence of environmental variables on the seasonal cycle of questing *I. ricinus*. Therefore, a 9-year time series of nymphal *I. ricinus* flagged at monthly intervals in Haselmühl (Germany) was compiled. For the first time, cross correlation maps were applied to identify optimal associations between observed nymphal *I. ricinus* densities and time-lagged as well as temporal averaged explanatory variables. To prove the explanatory power of these associations, two Poisson regression models were generated. The first model simulates the ticks of the entire time series flagged per 100 m², the second model the mean seasonal cycle. Explanatory variables comprise the temperature of the flagging month, the relative humidity averaged from the flagging month and 1 month prior to flagging, the temperature averaged over 4–6 months prior to the flagging event and the hunting statistics of the European hare from the preceding year. The first model explains 65% of the monthly tick variance and results in a root mean square error (RMSE) of 17 ticks per 100 m². The second model explains 96% of the tick variance. Again, the accuracy is expressed by the RMSE, which is 5 ticks per 100 m². As a major result, this study demonstrates that tick densities are higher correlated with time-lagged and temporal averaged variables than with contemporaneous explanatory variables, resulting in a better model performance.

**PP 13**

**Sentinel surveillance to assess plague risk indicators in Antananarivo, Madagascar**

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Plague is still a serious health problem in Madagascar. Endemic foci have been continued to expand and spatiotemporal variability in the distribution of human plague has been observed. Plague affects mainly the rural areas in the central highlands of Madagascar with the main reservoir *Rattus rattus*. In 2017, pneumonic plague and some cases of bubonic plague have been reported in urban area of Antananarivo the capital of Madagascar. The response strategy provided by the national plague control plan was implemented, targeting rats and their fleas, but no plague indicators was available to guide the field actions.

In order to develop strategies for control and prevention of plague, this study aimed to detect the circulation of plague bacillus *Yersinia pestis* in rodent and their flea in the city of Antananarivo and to determine the susceptibility of rat fleas to insecticides that used routinely for plague vector control.

Animal sampling was carried out from October to December 2017 in 18 sites in Antananarivo including 4 public places (markets and bus station) where people regularly move for purchase or travel. A total of 60 cage traps were placed at home per site for 3 consecutive nights. Fleas were collected from animals, identified and reared for insecticide tests and blood and spleen samples were removed to detect the presence of *Yersinia pestis* and antibodies IgG anti F1. Fleas were tested by PCR for *Y. pestis*. Eleven flea populations were tested for the susceptibility to organophosphates and pyrethroids.

A total of 392 animals were caught belonging to three species of rodents (*Rattus norvegicus, Rattus rattus and Mus musculus*) and one species of shrew (*Suncus murinus*). *R. norvegicus* was the most abundant at all sites. A total of 906 fleas were collected from all animals. Two efficient vectors of *Y. pestis* *Xenopsylla cheopis* and *Synopsyllus fonquerniei* were identified. Flea index varied from 0.2 to 8.9. No evidence of *Y. pestis* was found either in fleas or in rodents. However, 4 *R. norvegicus* captured on the markets found positive for plague antibodies. It means that the plague has circulated in rodent populations. All fleas population tested were resistant to pyrethroids whereas 3 populations were susceptible to organophosphate the current insecticide used to control fleas.

All these results call for vigilance in public health policies and rodent-flea control campaigns to prevent epidemic emergence of the plague in the city.
**PP 14**

**Epidemiology of Influenza virus in the Tunisian Military Hospital between 2017 and 2018**

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**Introduction:** Influenza illness is a serious public health problem since it causes significant morbidity and mortality, especially in vulnerable individuals. The objective of our study was to describe the epidemiological profile of confirmed Influenza virus cases sent to virology laboratory of the Tunisian Military Hospital (TMH) during the year 2017-2018.

**Methods:** A cross-sectional epidemiological study including all patients referred to the virology laboratory of TMH for exploring Influenza virus was done between 30/11/2017 and 22/04/2018. For each patient a letter with clinical information was analyzed. The variables studied were age, sex, comorbidity. For each patient we performed a respiratory sampling to be immediately analyzed by immuno-chromatographic kit “BioNexia influenza A + B”. Qualitative variables were expressed in numbers and percentages. The percentages were compared by the chi-square test.

**Results:** During the study period, we collected 38 patients with positive Influenza virus test among the 161 suspected cases. More than half of the patients (76.3%) were infected with Influenza A virus. The epidemic peak was reached in the 51st week of 2017 (8 positive cases). The most vulnerable age categories were 3-15 years (55.3%) and >60 years (23.7%) without predominance of sex (sex ratio=1). The confirmed cases of influenza were mainly from the pediatric service (60.5%) and from emergencies (26.3%). All patients were symptomatic on examination: influenza-like illness and fever were present respectively in 34.8% and 29.8% of cases. Nearly one of five patients (26.31%) had co-morbidities: diabetes was the most common co-morbidity (30%). One patient died from influenza A infection during the study period. The viral infection was not significantly associated with either sex (p = 0.26) or with the presence of comorbidity (p = 0.65). A statistically significant association between age and viral infection was found (p = 0.003).

**Conclusion:** The present study has highlighted the predominance of Influenza A virus type among the cases infected during the influenza epidemic in the TMH between 2017-2018. Anti-influenza vaccination of subjects at risk is essential for primary prevention against severe forms of influenza, hence the importance of sensitization to improve the vaccination coverage rate.

**PP 15**

**A new geographical area on the map of Crimean-Congo hemorrhagic fever virus: first serological evidence in the Hungarian population**

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The Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne viral disease causing various symptoms (from asymptomatic infection to severe hemorrhagic manifestations) with a potential fatality rate of up to 30% among hospitalized. The main vector of the virus is the *Hyalomma marginatum* tick with an expanding distribution in Europe. The virus is endemic in Africa, Asia, the Middle East and the Balkan region of Europe, however autochthonous cases were also reported from Spain in 2016. Due to climatic changes the Hyalomma tick is spreading northward and was first reported in Hungary in 2012. Despite vector presence and limited serological evidence among the animal population (European brown hare), no cases have been reported in Hungary so far. The aims of study were to set up the first comprehensive and systematic pilot surveillance and to assess the prevalence of CCHFV specific IgG based on a retrospective panel of 2700 serum samples obtained from voluntary blood donors by the Hungarian National Blood Transfusion Service between 2008 and 2017. For the pilot surveillance the whole-virus containing immunofluorescent slides (IFA) were produced and verified under BSL-4 conditions at the National Biosafety Laboratory, National Public Health Institute, Budapest, Hungary. For confirmation a commercial ELISA kit was used. We found 12 anti-CCHF IgG reactive samples from 8 statistical regions of Hungary. The most affected areas are the western and central regions, with the highest rate of positivity found in Jász-Nagykun-Szolnok region (2.97% prevalence) in the Great Plain area of central Hungary. Male donors were most likely to be affected (0.62% prevalence), and we found that most of the seropositive samples arisen from the 18-35-year age group. This is the first validated serological evidence of CCHF infection in the Hungarian population. Considering
our results, it would be strongly recommended to set up a comprehensive serosurveillance program focusing on both the human and animal population, and also to evaluate the distribution of the ticks in Hungary. Our results attach great importance to increase the awareness of clinicians and other at-risk populations about the potential emerging threat of CCHF.

PP 16
Hemorrhagic Fever with Renal Syndrome (HFRS) in Poland

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Hemorrhagic fever with renal syndrome (HFRS) is an acute viral zoonosis occurring due to the hantavirus infection. On the territory of Poland it constitutes the only case of viral hemorrhagic fever. The infections occurring in Poland are mainly caused by the Puumala and Dobrava serotypes. The aim of the study was to confirm the laboratory infections of Hantavirus in patients suffering from acute renal failure, presenting specific symptoms of hemorrhagic fever. Patient interview revealed that all patients had contact with Hantavirus environment through infectious animal vector.

The study covered 5 patients with renal syndrome from the Lublin and Masovian Voivodeship. Laboratory tests were conducted in Medical Diagnostics Laboratory of the Regional Sanitary and Epidemiological Station in Rzeszów. The presence of the classes IgM and IgG specific antibodies directed against the nucleocapsid structural protein was proved through the application of serologic method called ELISA screening.

The conducted study showed that in the case of two patients there were high titres of IgM class of 3,57 and 7,25 and above 200 RU/ml in the IgG class. These results may indicate an ongoing hantavirus infectious processes. In one case the level of marked antibodies in the IgM class was doubtful and the level of the antibodies in the IgG class was above 200 RU/ml. In another case only the high titre of the IgG class was indicated at the level of 190,53RU/ml which may indicate a past contact with the virus and a past infection. In one case there were no antibodies indicating contact with Hantavirus which implies no past contact with this viral factor. In the case of all patients included in the study, an additional follow-up indirect immunofluorescence test should be conducted.

All the carried out analyses proved that Poland isn’t a country free from the hemorrhagic fever virus. It is particularly important in the case of people exposed to direct or indirect contact to rodents. This group includes such professions as zoologists, foresters, rural population and tourists as well as soldiers performing their activities on training ground or in forest areas.

In view of literature and the significant occurrence of Hanta antibodies in patients included in the risk group, it may be claimed that the diseases caused by Hantavirus constitute a significant factor influencing the medical status of the group in focus all over the country.

PP 17
CCHF and RVF virus infections in animals in Egypt

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Rift Valley fever (RVF) is a hemorrhagic fever infection of domestic ruminants and humans caused by an arbovirus belonging to the Phlebovirus genus (family Bunyaviridae). It causes high mortality rates in newborn ruminants and abortion in pregnant animals. Infections in humans are typically associated with self-limiting febrile illnesses. However, in 1% to 2% of affected individuals, infections can progress to hepatitis, encephalitis, retinitis and hemorrhagic syndrome. RVFV is transmitted primarily by mosquitoes but can also be transmitted by direct contact with infected tissues and fluids. Traditionally, RVF virus has been restricted to sub-Saharan Africa, but was detected in Egypt in 1977, where it was the cause of a massive epidemic-epizootic. Up to then, RVF had been seen largely as a veterinary problem.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a member of the genus Nairovirus (family Bunyaviridae). The spread of CCHFV primarily coincides with the distribution of Hyalomma ticks as its main vector. It is described in parts of Africa, Asia, Eastern Europe, and Middle East. In Egypt serologic surveys revealed evidence of CCHFV antibodies in ruminants, but no human case was reported yet. Animals do not show clinical signs, whereas infections of humans can cause a severe
hemorrhagic fever with high lethality rate. Humans become infected through the bites of ticks or by contact with viremic patients or livestock. RVFV and CCHFV are listed as a select agent with significant potential for international spread and use in bioterrorism.

One part of this project is the determination of the prevalence of RVFV and CCHFV in Egypt by investigation of livestock and mosquitoes. Therefore, 2500 collected samples of ruminants will be tested by molecular and serological methods. Vectors will be trapped in sites, where high seroprevalence in animal hosts is detected, and will be species-determined and as well tested molecularly. The results will be used to define risk factors and develop plans for preventive and control measures. Furthermore, by organizing workshops for scientists and employees of our Egyptian partner laboratories about diagnostic and biosecurity as well as Good Laboratory Practice, the competence of handling agents of high biosafety level will adjust to international standard. Therefore, raising awareness and enhancing level of knowledge in detection and diagnostic will lead to increased biosecurity and biosafety in Egypt.

PP 18
Genetic analysis of Rhipicephalus sanguineus s.s. from Canary Islands and Cyprus based on mitochondrial DNA sequences

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The aim of this work was to determine the evolutionary relationship among tick populations of Rhipicephalus sanguineus sensu lato distributed in Canary Islands and in Cyprus and different lineages of R. sanguineus s.l. distributed in different regions of Sub-Saharan Africa, America and Europe. DNA sequences of two mitochondrial genes were analyzed. Ninety-eight 16S rRNA gene sequences from Canary Islands and 18 from Cyprus of R. sanguineus s.l. were obtained. Phylogenetic analyses were performed including different lineages of R. sanguineus s.l. from America, Europe and Africa, and species belonging to the R. sanguineus group as Rhipicephalus camicasi, Rhipicephalus guilhonii, Rhipicephalus rossicus, Rhipicephalus pusillus, and Rhipicephalus turanicus. One lineages of R. sanguineus s.l. is living in Canary Islands which cluster with the “temperate lineage” sequences from North and South America and Western Europe. One mitochondrial lineage of R. sanguineus s.l. present in Cyprus to R. sanguineus s.l. ticks from south-eastern Europe (Romania, Turkey and Greece) and the other one is close to R. sanguineus s.l. and R. turanicus s.l. from China. Both evolutionary entities are clearly different to the evolutionary entity formed by R. sanguineus s.l. from western Europe and temperate areas of America, which probably represents the taxon R. sanguineus s.s.

The taxonomic status of these taxa will remain unresolved until new lines of evidence become available to complement the current results based on mitochondrial DNA.

PP 19
Investigation of tick-borne encephalitis spread in the non-endemic regions of Kazakhstan

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The endemic tick-borne encephalitis (TBE) areas are located in the south-eastern part of Kazakhstan (Almaty and East-Kazakhstan regions, Almaty city). However, in recent years the incidence of TBE in non-endemic areas has been reported. In 2010-2017, in Akmola region 24 human cases of TBE have been identified. For 7 months of 2018, there were reported 2 human cases of TBE in the North-Kazakhstan region. The main vector of TBE virus (TBEV) is I. persulcatus. Ticks infection index for I. persulcatus in East-Kazakhstan is 2.8%, in Almaty region and Almaty city 3.3%. All isolates after genotyping belonged to the Siberian subtype of TBE virus. During the last years, we investigated ticks from non-endemic areas for the presence of TBEV and determining their epidemiological role. In 2012-2013,
278 *D. marginatus* ticks from the North-Kazakhstan region were examined by ELISA. Ticks infection index for TBEV was 8.2% (23 samples). In 2012, by using RT-PCR were tested 347 ticks from the North-Kazakhstan region, mainly from the Dermacentor genus. RNA of TBEV was determined in 15.9% of ticks: *D. marginatus* - 9.0%, *D. reticulatus* - 34.8%. In 2013, 141 *D. marginatus* from the Karaganda region were tested by RT-PCR. RNA of the TBEV was detected in 44.7% of tick samples. In addition, by using ELISA method, we tested sera samples from 125 persons with tick bites in the North-Kazakhstan region in order to detect IgM/ IgG for TBEV. 4 sera samples were positive for TBEV IgM (3.2%) and 3 sera samples for TBEV IgG (2.4%).

In 2017, within the framework of the TAP-10 project on the investigation of TBE epidemiology in the northern regions of Kazakhstan, we tested 257 tick pools (2036 ticks). The TBEV positive tick samples in RT-PCR were 9.3%, including in the next regions: North-Kazakhstan region – 15%, Akmola region – 9.7%, Kostanay region – 5.1%. Positive tick samples were *D. marginatus* (62.5%), *D. reticulatus* (33.4%) and *I. persulcatus* (4.1%).

TBE morbidity and the results of serological and molecular studies on TBE among ticks and population with tick bites in the northern Kazakhstan allow us to assume that the boundaries of the TBE natural foci extend to northern Kazakhstan and the main TBE vector belongs to Dermacentor ticks. The positive results of tick studies in Karaganda region (central Kazakhstan) also confirm the activity of one more likely a TBE natural focus.

**PP 20**

Periodic screening for tick-borne diseases

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Lyme Borreliosis (LB), also known as Lyme disease, is a multi-systemic inflammatory disorder caused by an infection with bacteria of the genus *Borrelia*. These bacteria are transmitted to humans via tick bites. LB is one of the most frequent infectious diseases worldwide. This disease is relatively easy to cure when detected in an early stage. If left untreated, infection can spread to the joints, heart and nervous system, causing severe complications via a large variety of symptoms, which may persist over months or years.

Unfortunately, an accurate diagnosis at the early stage is difficult using the currently available tests. Many early LB patients are missed. As a consequence, effective treatment of LB is significantly hampered. If the disseminated form is left untreated, it may lead to severe disability of the patient. It also results in very high long-term costs and large impact to families, health care systems and society as a whole. The development of accurate diagnostic paradigms for LB in an early stage is of utmost importance.

Besides developing novel tests for LB, Innatoss has developed a screening program for anyone at high risk of contracting LB. The program targets organizations in green maintenance, forestry and water management. Participants range from golf clubs to cemeteries. By providing an annual screening at the end of the tick season, employees that have been exposed are identified and can be treated.

The key feature of the program is that it is based on multiple *Borrelia* ELISAs and that it starts with a baseline measurement to identify existing antibodies. The baseline measurement facilitates interpretation of subsequent tests.

The program currently covers > 1500 people, with more than half having been tested at least 2 times. Prevalence of prior infection is 19-39 % in this high-risk group. We demonstrate that on average 2% of the employees pick up an infection without noticing the red rash.

For the future, we believe that expanding this screening program to other Tick-Borne Diseases will provide not only additional protection for green workers by identifying subclinical infections, but will also provide a systematic survey of antibody prevalence for LB and co-infections in different regions and risk groups and an evidence-based method for evaluating tick bite prevention measures.
Q fever fatigue syndrome (QFS). Sporadic military outbreaks have been described since World War II & sero-surveillance showed that 1.7% of approximately 10,000 British troops in Helmand, Afghanistan were infected during 6-month deployments from 2008-11. During this campaign, clinical guidelines recommended early empirical treatment of non-malarial UFI cases with doxycycline 200mg daily for 2 weeks to cover acute Q fever & (rickettsial infections) & then clinical follow-up at the UK Role 4 military healthcare facility.

From 2008-2015, there were 85 British military cases of acute Q fever identified from Helmand, Afghanistan. A retrospective study of demographic, occupational, clinical, laboratory & treatment factors was performed to identify the common features of acute Q fever & risk factors for developing chronic Q fever or QFS (because these complications will usually lead to a medical discharge from military service). Eighty-four cases (99%) were male, the median age was 28 years and Research), Academic Department, Birmingham, Porton Down, Salisbury, UK.

Of the 85 cases, 59 patients (69%) were smokers compared to the 34% reported in British Army male soldiers in 2013. Doxycycline 200mg daily for 2 weeks was given to 70 cases (81%) and the median time from onset of symptoms to receiving doxycycline was 5.5 days (range 0.3-324 days). Two cases (2%) developed chronic Q fever & 19 (22%) developed QFS. Binomial logistic regression showed that QFS was more likely if empirical doxycycline treatment was given >5 days after the onset of symptoms (OR 2.9, 95% CI 0.95-8.91, p = 0.062), but due to a low sample size this result did not reach statistical significance. No effect was seen with the other risk factors.

Smoking may be a risk factor for developing acute Q fever (which has been described previously) & delayed empirical antibiotic treatment may be a risk factor for QFS.

PP 22

Coxiella burnetii can be found in cellular debris in the semen, but is not attached to the spermatozoa during acute infection in the A/J mouse model

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Q fever is caused by Coxiella burnetii, a Gram-negative bacteria found in the birth products of ruminant animals such as goats, cattle and sheep. Inhalation of the bacteria is the most common route of human infection. There are reports in a US serviceman and Polish Shepherds with acute Q fever hypothesising the potential risk of sexual transmission to their female partners. In this study 30 A/J mice were challenged with an aerosol of Phase 1 C. burnetii Nine Mile strain. They all became unwell demonstrating clinical signs and weight loss from day 4 to day 11 post challenge. Groups of 5 mice were culled at set time points post challenge (day 5, 8, 12, 15, 19 & 35) to look for bacterial dissemination to multiple organs sites including the semen and spermatozoa.

Semen was cultured from all samples on ACCM-2 plates in quadruplicate and viable counts observed in 20% of mice at day 5 and 40% of mice at day 8, all other semen samples showed no detectable growth. Previous literature has suggested using electron microscopy that C. burnetii attaches to the head of the sperm and could therefore be transferred passively to female sexual partners1. In our study the sperm samples from each mouse were stained using an anti Cox-LPS antibody and a DNA stain and reviewed under a confocal microscope. In contrast to uninfected mice, cellular debris could be seen over the head and midpiece of the sperm but with no associated bacteria. C. burnetii could be seen in adjacent cells under microscopy from the 2 mice with viable growth at day 8.

It is therefore postulated that the mode of sexual transmission is not via passive transfer with the sperm but through C. burnetii transmission in cellular debris in the semen. Little published literature exists on sexual transmission of C. burnetii. It does appear in this study that the bacteria is not readily disseminated to the semen and therefore there is a low likelihood of sexual transmission.

References:

PP 23

From Q fever control to psittacosis prevention

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References:
The Q fever epidemic in the Netherlands, from 2006 to 2010, accumulated in 4,250 notified human cases, 749 hospitalised patients, 26 direct and 74 indirect deaths and an estimated number of more than 40,000 infected persons. The outbreak is considered as the biggest Q fever outbreak ever reported worldwide. Dairy goats and dairy sheep were initially suspected and later on confirmed as the source of the outbreak. This resulted in drastic control measures including culling of 58,150 pregnant high risk goats on Q fever positive farms and 848,906 vaccinated dairy goats and dairy sheep nationwide.

Key in the effective control of this Q fever outbreak was the identification of the source of the human outbreak by the molecular confirmation of the epidemiological links between human cases and goat farms. This, however, was time consuming, causing a delay in the implementation of effective control measures. In addition, the lack of data-exchange between the human public health and veterinary domain hampered the control of the outbreak as well. Lessons learned from this outbreak are that for a timely response in zoonotic outbreaks such as Q fever, a prompt exchange of data between the human and animal health chains is vital.

We applied these lessons on a comparable to Q fever zoonotic disease: psittacosis. Psittacosis, caused by Chlamydia psittaci, is an intracellular growing bacterium that can survive in the environment. C. psittaci can infect a wide range of bird species as well as humans. Humans become infected via inhalation of dried contaminated bird excreta. Known risk factors for human psittacosis are direct contact with hobby birds, occupational exposure to birds, and an indirect relation with poultry. The aim of our research is to reduce the disease burden caused by psittacosis and to add essential information on C. psittaci in humans and animals. Therefore we established a ‘One Health’ data exchange platform for psittacosis that facilitates connecting human cases to animal sources via clinical, epidemiological and genotyping data. A first assessment of psittacosis in Dutch layer farms indicate that C. psittaci is not present on these farms although exploratory spatial analysis of human psittacosis notifications in the Netherlands showed a large cluster that covered a highly poultry-dense area but also the presence of additional clusters in areas that had a low poultry density. Additional research is needed to understand these two findings.

**PP 24**

**Epidemiological features of tularemia in Kayseri / Turkey**

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**Introduction and Aim:** Tularemia is a zoonotic disease caused by Francisella tularensis. In recent years tularemia has become a threat for public health and spreading the most part of Turkey, especially Central Anatolia Region at 2009-2010. Kayseri is a city in Central Anatolia, Turkey. The aim of the study was to examine the epidemiological characteristics of tularemia cases reported from Kayseri between 2010-2017.

**Material Methods:** For this cross-sectional descriptive study, data were obtained from Kayseri Provincial Health Directorates’ records with their permission in April 2018. All the reported tularemia cases residing in Kayseri (n=154) between 2010-2017 years were evaluated. Serum samples taken from suspected cases were sent to Turkey Ministry of Health National Reference Laboratory for the diagnosis of tularemia. Clinical diagnosis was confirmed by microagglutination test (≥1/160 titers). Tularemia cases were evaluated in terms of age, sex, place and time at computer; in addition, tularemia morbidity rates of Kayseri and Turkey were compared.

**Results:** Total of 154 tularemia cases were reported between 2010-2017 in Kayseri. Kayseri has 16 districts, cases were reported from 13 districts and no tularemia cases were reported from other 3 districts during eight years. Highest rates of morbidity were found in three northern most neighbor districts; Ozvatan, Sarıoglan and Felahiye. Most of the patients (56.5%) were residing in rural areas. Year with the highest case report was 2011 (40% of all cases). Tularemia cases were reported at all months, but the highest rates were at February (26.1), March (19.0%) and January (15.7%) while the lowest was at June (%1.3). The mean age of the cases was 41.0 ± 21.6 (min = 1, max = 86) years. Childhood cases were 15.7% of all cases and largest numbers of cases were in the 30-49 age group (27.5%). Morbidity rates were higher in women and over 65 years of age. In the majority of positive
cases, microagglutination titers were found to be between 1/320-1/1280 (79.1%). Kayseri tularemia morbidity rate was higher than Turkeys’ between 2011-2016 years. One death due to tularemia was reported in the evaluated eight years.

**Conclusion:** A tularemia outbreak was initiated in Kayseri at 2010. Most cases were seen between 2010 and 2012. After than the cases were decreased but not disappeared. We believe that climate changes and the vector’s biology have an important role on life cycles of *F. tularensis* in the environment.

**PP 25**

**Sustainability of protein pXO2-60 for serological studies to determine the prevalence of Bacillus cereus biovar anthracis in Côte d’Ivoire**

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Anthrax is endemic in most countries of sub-Saharan Africa, but official reports are rare. In Côte d’Ivoire, anthrax caused by *Bacillus anthracis* (Ba) is known to be endemic in the border regions next to Burkina Faso and Ghana. However, outbreaks in livestock or humans are rarely noticed and recorded. Atypical *B. anthracis*-like bacteria now designated as *B. cereus* biovar anthracis (*Bcva*) were isolated from wild great apes and other mammal species that had died of an anthrax-like disease in the Taï National Park in the south-western region of Côte d’Ivoire. Until now, no human cases of *Bcva* were reported, but exposition of the human population is very likely due to hunting and consumption of bush meat. Retrospective seroprevalence studies can be performed using the immune response against the protective antigen PA, which is produced by both *Ba* and *Bcva*. To detect specific antibodies against *Bcva*, we determined the suitability of the protein pXO2-60 which is only secreted by *Bcva* and not observed in *Ba* due to a mutation in the sequence for the signal peptide. The protein was cloned and overexpressed in *E. coli* to obtain a recombinant protein that can be used to establish serological assays. Only mice immunized with culture supernatants of *Bcva*, but not *Ba* produced antibodies against recombinant pXO2-60. In addition, antibodies against pXO2-60 were not detected in sera from patients after infection with classic *Ba*, confirming suitability of the antigen for specific detection of anti-*Bcva* antibodies.

We conducted a serological study on human sera from the area surrounding Taï National Park to determine the antibody prevalence against *Ba* and *Bcva*. Screening was performed using anti-PA-ELISA, and positive sera were analyzed by Western blot with small membrane stripes loaded with PA and pXO2-60. Analysis of approx. 1400 sera revealed a seroprevalence of anti-PA antibodies of up to 38% (average 22.4%) in humans in the Taï area, varying in the different villages investigated. Additionally, up to 19% (average 10.5%) of human sera were tested positive for antibodies against pXO2-60, indicating an infection with or exposition to *B. cereus bv anthracis*.

**PP 26**

**Human anthrax in Kyrgyz Republic: Epidemiology and clinical features**

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**Background:** Today, Anthrax is still important issue in Kyrgyz Republic as various factors such as: presence of persistent soil sources of anthrax, development of livestock breeding forced home-slaughter without following veterinary and sanitary rules, sale of meat products without the knowledge of Veterinary Service specialists. Human anthrax cases were reviewed in one center.

**Methods:** The data of 234 cases of anthrax in the southern regions of Kyrgyz Republic were recorded prospectively. Epidemiology and clinical features of the infection and effectiveness of antibiotic therapy in patients were reviewed.

**Results:** Among the patients, men in the group age of 31-50 prevailed. The infection episodes were registered mostly during the summer and autumn. The analysis of epidemiological data shows that anthrax contamination occurred as the result of direct contact with farm animals: forced home-slaughter, butchering and meat sale. Cutaneous lesions are localized mostly in upper extremities. Among the observed patients mild form of the disease prevailed. *B. anthracis* was susceptible to amoxiclav, ofloxacin, cephalosporins of III generation, doxycycline, ciprofloxacin and rifampicin, penicillin, streptomycin; it was resistant to ampicillin, gentamicin, chloramphenicol.

**Conclusions:** Anthrax is still a public health problem in rural areas, particularly in southern regions, in Kyrgyzstan. The future studies should be fo
cused on surveillance and notification of the disease, education of the risk groups, animal health care, infection control and prevention of the disease and to set up collaboration with neighboring countries.

**PP 27**
WITHDRAWN

**PP 28**
The role of rodents in the persistence of anthrax soil foci

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**Introduction:** Anthrax is a zoonotic disease caused by Bac. anthracis. A causative agent of anthrax has different phases of existence, and one of them is in the soil. The rodents carrying out the digging activity are a part of the chain of a pathogen circulation. According to the previous studies B. anthracis was isolated from rodents.

**Aim of investigation:** The investigation of anthrax infection in rodents in natural foci for further investigation their possible role in an epizootic process and in a circulation of the pathogen in the nature.

**Materials and methods:** The genetic methods of investigation were used.

**Results:** The investigation of rodents was conducted in Almaty oblast. Annually anthrax cases among humans and animals are registered in this territory. 430 wild rodents were investigated for the presence of anthrax DNA. The DNA of anthrax was detected in three samples of rodents’ vitals. Two samples included the vitals of house mice collected near “Koksu” village. Two cases of anthrax among domestic animals near “Koksu” village were registered in 1966 and 1972. The third sample included the vitals from house mice collected in houses of “Rudnichnyi” village. The cases of anthrax among domestic animals were also registered in the surroundings of this village in 1972, 1978 and 1986. The soil digging is a significant aspect of rodents’ lifecycle. Due to the soil digging the rodents are infected by Bac. anthracis in the territory of anthrax foci. The distribution of Bac. anthracis by rodents could contribute to the soil recontamination and the expansion of Anthrax foci.

**Conclusion:** Rodents are «silent hosts» of Bac. anthracis and an indicator of anthrax soil foci activity. The investigation the rodents’ infection in natural foci by the screening the animals could help understand the role of possible role in the epizootic process among animals.

**PP 29**
Lethal outcome of the first case of oropharyngeal anthrax reported in Georgia, 2017

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**Background:** Although, anthrax is not reported in developed countries, its research is still essential due to the threat of bioterrorism. As in other developing countries, anthrax is an endemic disease in Georgia. Cutaneous anthrax is a most common form registered in Georgia as it is in other countries. Our goal is to describe the first oropharyngeal anthrax case in Georgia thus increasing awareness of physicians.

**Case report:** A 28-year-old woman presented with initial symptoms of hyperthermia (39°C), vomiting, headache, sore throat, difficulty in swallowing and swelling of the mandibular glands. Patient sought for medical care at the 4th day after the disease onset and was admitted to the hospital in severe condition with respiratory insufficiency, tachycardia, low blood pressure, swelling of the neck and on the upper third of the chest, glands and lingual hypertrophy, hard removable grayish membrane on the right side of the soft palate that was bleeding upon removal and malodorous specific smell from the mouth. Consequently, the patient has developed a generalized seizure attack with desaturation that was managed with artificial respiration. Initially, diphtheria was suspected, but B. anthracis was confirmed by qPCR in the pharyngeal smear and isolated by culture. Ulcero-necrotic oropharyngeal lesions with following satellite ulcers were developed on the 6th day of the illness. Consequently, the patient manifested with cerebral edema, infectious toxic shock, coma, pneumonia, bilateral pleuritis and ascites. Antibiotics therapy was initiated with PenicillinG, continued with Ciprofloxacin the following day, which was replaced by Meropenem. Epidemiological investigation revealed a contact with raw ground beef.

**Conclusion:** The first confirmed case of ortho-
ryngeal anthrax was reported in Georgia. Late sicking medical care and delayed treatment most likely contributed to lethal outcome. This emphasizes an importance of timely seeking medical care, early diagnostics and timely initiation of treatment. We recommend considering anthrax in patients with severe pharyngitis as a part of differential diagnosis.

PP 30
Effect of environmental cues on Bacillus anthracis soil populations

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In the soil environment, the bacterium Bacillus anthracis exists as a dormant spore, waiting for the necessary signals to permeate through the exosporium layer and initiate germination. Vegetative organisms are sensitive to most disinfectants and heat but when exposed to air they can sporulate quickly. The spore surface, or exosporium, is coated with glycoprotein that is involved in spore binding to environmental surfaces, generates spore hydrophobicity and affects spore germination. Spores contact a host through ingestion, inhalation, or cutaneous inoculation then germinate to the vegetative form and elaborate the A/B-type anthrax toxin made up of protective antigen (PAG), lethal factor (LF), and edema factor (EF) which combine and ultimately cause host death (typically grazing mammals). Outbreaks still occur globally, including areas where vaccination reaches livestock but not wildlife. Outbreaks are epidemic with pronounced seasonality. In the mid-latitudes, such as the United States and Australia, outbreaks have long been associated with early wet springs followed by hot, dry summers. By using remotely sensed vegetation indices (e.g. NDVI) as a proxy for rainfall the anecdotal evidence does have a firm basis in reality. Earlier vegetarian green-up, an earlier start to spring, is correlated with epizootic outbreak years and increased severity of anthrax outbreaks during summers in the enzootic zone of Texas and the Australian Anthrax Belt. Major summer rain events tend to immediately precede the onset of anthrax cases in an outbreak. In Ghana, Namibia, and Zambia anthrax cases follow dry weather after rainstorms. It has been shown that animal behavior and herd population dynamics also affect outbreak severity. Our work investigates the effect of rainfall events on B. anthracis population flux in the soil, an often-overlooked aspect. Bioluminescent reporter strains were engineered and utilized in soil microcosm models to understand the effects of simulated environmental cues, such as temperature and rainfall among others, on B. anthracis sporulation, germination and vegetative growth. We have also investigated the effect of amoeba (Acanthamoeba castellanii) predation on B. anthracis sporulation, germination, and vegetative growth in soil microcosm models. This work sheds light on B. anthracis behavior in the soil following environmental cues and adds to the evolving knowledge of the drivers of anthrax outbreaks.

PP 31
Continuous isolation of tick-borne encephalitis virus from adult Dermacentor reticulatus ticks in an endemic area in Germany

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The tick-borne encephalitis (TBE) virus is transmitted to humans and animals through tick bites and is thought to circulate in very strict outlined natural environments called natural foci. The most common tick serving as vector for TBE virus in Central Europe is Ixodes ricinus, rarely it is found in other tick species and, so far, only in Poland in Dermacentor reticulatus tick. Between autumn 2016 and spring 2018 TBE virus was detected eleven times in flagged adults D. reticulatus (n=1,534), while I. ricinus nymphs (n=349) tested positive for TBE virus only once. I. ricinus males (n=33) and females (n=30), as well as five I. inopinatus (2 females, 3 males) and 14 Haemaphysalis concinna (3 females, 11 nymphs) tested negative for TBE virus by means of real-time RT-PCR. TBE virus was not detected in I. ricinus during the summer, when D. reticulatus was not active. Sequence comparison of the entire E gene of the isolated virus strains resembled each other with only 3 nucleotide differences. The most closely related viral sequences belong to TBE virus strains from Poland and Neustadt an der Waldnaab approximately 200 km east and 200 km south-west of the new focus. This is the first report of a TBE virus circulation in an endemic region where D. reticulatus and I. ricinus do occur sympatrically in nature but where D. reticulatus seems to play a key role in virus circulation.
PP 32

Migratory birds and dissemination of tick-borne pathogens: Crimean-Congo and Alkhurma hemorrhagic fevers, Rickettsia, and other pathogens

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To more clearly understand the ecology of tick-borne diseases, we investigated the role of migratory birds on the dispersal of ticks and tick-borne pathogens. During springtime northward migration periods in 2009-10 and 2014-15, we captured and released 36893 birds and collected 1771 ticks, at seven bird observatories in the northern Mediterranean basin. Tick specimens were homogenized, and nucleic acid extracted. Subsequently the samples were subjected to high thorough-put PCR screening methods for both an array of bacterial/parasitic pathogens and an array of viral pathogens. Species identification of the tick specimens was done by morphological as well as molecular methods. Most of the ticks (88%) were found to be of the Hyalomma marginatum sensu lato complex, presumably H. marginatum and H. rufipes. Rickettsia species from the Spotted Fever Group, mainly R. aeschlimanni, were detected in about half of the samples, Anaplasma in 3.5 % and Coxiella in 0,1 %. RT PCR screening also revealed Crimean-Congo Hemorrhagic fever (CCHF) virus in 3 ticks specimens and Alkhurma hemorrhagic fever (AHF) virus in 6. The birds infested by these ticks were all long distant migrants that winter in sub-Saharan Africa and breed in Europe. Presumably, the collected ticks had attached to the bird hosts prior to migration from Africa, as the all the observatories are located on where migrating bird likely first touchdown after crossing the Mediterranean. CCHF has recently appeared on the Iberian Peninsula, while AHF has emerged on the Arabian Peninsula during the past two decades and has also appeared sporadically on the western coast of the Red Sea. Our results indicate that transport of infected ticks could present a method of dispersal of both viral and bacterial tick-borne pathogens and partake in the maintenance of epidemic infection as well as the initiation of mammalian-tick infectious cycles in novel geographical regions. We also propose that surveillance of tick-borne pathogens emanating from endemic areas may also be done by monitoring bird migration.

PP 33

Tick-borne encephalitis virus of the Siberian subtype: Analysis of genetic diversity, geographical distribution and evolution

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The tick-borne encephalitis virus (TBEV), a member of the Flaviviridae family, is currently subdivided into three main subtypes—the European (TBEV-Eu), the Far-Eastern (TBEV-FE), and the Siberian (TBEV-Sib). The TBEV-Sib is the most common subtype and found in all regions where TBEV was detected, except for Western Europe. Currently, three genetic lineages have been described within TBEV-Sib.

In this study, detailed analysis of TBEV-Sib genetic diversity, geographic distribution, phylogeography and divergence time of different TBEV-Sib genetic lineages based on gene fragments, complete genome sequences, and all currently available data in the Genbank database was performed. As a result, a novel Obskaya and Bosnia lineages within the TBEV-Sib were identified. It was demonstrated that the Zausaev lineage is the most widely distributed among the TBEV-Sib lineages, and was detected in all studied regions except the Far East. The Vasilchenko lineage was found from Western Siberia to the Far East. The Baltic lineage is presented from Europe to Western Siberia. The Obskaya lineage was found only in Western Siberia. TBEV strains from a newly described Bosnia lineage were detected in Bosnia, the Crimean peninsula, Kyrgyzstan, and Kazakhstan. The greatest divergence of the TBEV-Sib genetic variants was observed in Western Siberia. Within the TBEV-Sib, the Obskaya lineage diverged from the common ancestor the earliest, after that the Bosnia lineage was separated, then the Baltic lineage, and the Zausaev and Vasilchenko lineages diverged the most recently. This study was supported by the Program of Fundamental Scientific Research of the State Academies of Sciences (project No. 55.1.1), research work №
Tick borne encephalitis virus (TBEV) is a tick-borne flavivirus causing flu-like illness and meningoencephalitis, some with severe clinical outcomes in the human host.

With approximately 10,000 to 12,000 clinical cases reported each year TBE is one of the most important viral infections of the central nervous system. Today TBE is endemic in Russia and 27 European countries and so has become an international public health problem.

There is an inactivated TBEV vaccine but no live vaccine or TBEV specific therapeutics.

TBEV strains differ in their capacity to enter the central nervous system of mice. Strain TBEV HB171/11 is significantly less neuroinvasive than strain Torö 2003 in the mouse model. These two TBEV strains differ only in 35 amino acid positions (deviating aa positions (daap) 1-35) across their respective open reading frames, four of them in the structural protein coding part of the TBEV strains.

The hypothesis underlying this project is that one or several of these amino acid differences determine neuroinvasiveness in the mouse. Based on the plasmids generated by Johannsson et al., we have changed corresponding amino acids in strain Torö to reflect the amino acid present in strain HB171/11 using site directed mutagenesis with the aim to generate an attenuated phenotype with reduced neuroinvasion. To test this hypothesis the mutated genome needs to be expressed in a single plasmid vector and infectious virus generated from full length genomic RNA transcripts. We are currently assembling several variants with mutations at daap 1-4 which are all located in the area of the genome encoding structural proteins and will report on progress.

This work provides the foundation for further investigation into the neuropathogenicity/neuroinvasion of TBEV and will give important insights for the development of a neuroattenuated live vaccine.

The aim of the study was to obtain the complex characteristics of the tick-borne encephalitis virus of European subtype (TBEV-Eu) circulating in Western and Eastern Siberia. Using the full-genome sequencing approach it was demonstrated that TBEV-Eu strains isolated in Siberia are genetically similar to the strains from the European part of its habitat range, and with the representatives from South Korea. It was confirmed that the homology of TBEV-Eu strains isolated in different parts of the virus habitat area from Scandinavian countries in the west to the eastern borders of the area (South Korea) is much higher than the homology level of TBEV strains of Far Eastern and Siberian subtypes. The Siberian population of TBEV-Eu is presented with two groups of strains called Eastern Siberian and Western Siberian variants, which differ in the combinations of amino acid substitutions in all proteins except NS2B protein. It was found that TBEV-Eu strains from Siberia possess high neurovirulence, but some of them, like strains from Europe, demonstrate low invasiveness. It was shown that TBEV-Eu strains have the good adaptive capacity, and therefore, can easily adapt to the circulation in various biocenoses in the territory of different landscape-geographical zones. It was found that the circulation of TBEV-Eu subtype was observed in Siberia territory for over 40 years. It was emphasized that in spite of circulation of TBEV-Eu subtype in the significantly different areas by climatic conditions, topography, landscape, habitat characteristics, it possesses a high degree of genome stability.
**PP 36**

Characteristics of the Baikal subtype of tick-borne encephalitis virus circulating in Eastern Siberia

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During the study of the genetic variability of the tick-borne encephalitis virus (TBEV) in Eastern Siberia, a group of 22 strains with a unique genetic structure significantly different from all known TBEV subtypes was identified. This TBEV variant was tentatively called «group 886». So, for this original TBEV group, it was necessary to study its genetic and biological properties, clarify its TBEV taxonomic status, its range, evolutionary history, etc.

**Aim:** The generalization of the currently available data on genetic and biological properties of TBEV «886 group».

**Materials and Methods:** The genetic structure of «group 886» strains was studied by the complex of molecular-genetic methods (MHNA, sequencing of fragments or the complete genome).

**Results:** It was shown that «group 886» strains form a separate cluster on the phylogenetic tree, and the level of genetic differences from other genotypes is more than 12%. It was defined that this TBEV variant has its own habitat area (Irkutsk region, Republic of Buryatia, Trans-Baikal region, Northern Mongolia). Its ecological connection with all links of the transmissive chain (ixodid ticks, small mammals, humans), participation in human pathology, stability and duration of circulation in the Baikal region, individual evolutionary history were proved. Some phenotypic characteristics of the «group 886» strains were considered.

**Conclusion:** The presented data testify to the validity of the «886 group» isolation as an independent genetic type. Taking into account the geographical distribution of this TBEV genotype, we propose to assign it the name «Baikal genotype/subtype».

**PP 37**

The risk occupation of TBE in Mongolia

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**Background:** Eleven suspected TBE cases were hospitalized in 20 June from 267 firemen of emergency management agency who came from Ulaanbaatar, Dundgobi, Uvurkhangai, Orkhon and Dornogobi provinces in Selenge. They were worked in fire in Eruu, Mandal soums of Selenge province from 17 May to 12 June 2018. We conducted survey to verify diagnosis and determine risk of infections.

**Method:** Face to face interview with 111 firemen who worked in Selenge province in June were conducted. And blood samples were taken from 24 people who had symptoms.

**Result:** Totally, 96% of them male and 20–48 years old (average age 32.2) firemen were enrolled. They have been working in emergency management agency for 1 to 28 years. 61.3% of them had vaccinated against TBE. 46%(43) of them get tick bitten, when they were damping the fire in Selenge province from May to June. Twenty (38%) of them were get tick bites once, 23(44%) of them were get tick bites two times, 8(15%) of them were get tick bites three to four times and one fireman was get tick bites eight times.

An around 24 (53%) firemen were developed following symptoms after tick bites. Including headache-48.1% (13), first affect-18.5%(5), fever-14.8% (4), muscle aches-14.8% (4), vomit-7.4% (2), dizziness-7.4% (2), eye hole aches-7.4% (2), glandule-3.7% (1) and stiff neck-3.7% (1). There are 5 (20.8%) TBE cases were confirmed. To increasing tick bite times are higher risk (r=0.83) to develop symptoms among firemen when they were damping the fire in Selenge province.

**Result:** The risk occupation of TBE in Mongolia. 48.6% of firemen answered need to vaccinated, 30.6% of them need to check tick in body and clothes every 2 hours, 24.3% of them must wear protective clothes and 7.2% of them answered never use protection against tick bites. Most of them getting knowledge due to health organization (60.4%) and by the internet (42%) and following TV (32.4%) and training materials (15.3%).

**Conclusion:** Fireman is becoming one of the risk occupations of TBE when firebreak out in TBE risk forested area in Mongolia. Therefore, vaccination coverage and training activities among firemen in emergency management agency need to enhance.
Diagnosis of human *Brucella* in Southern Morocco by IgM ELISA, Rose Bengal Test and real-time PCR

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Brucellosis is a zoonotic infectious disease caused by different species of the genus *Brucella*. The disease is debilitating, often chronic and insidious, and associated with serious complications (e.g. endocarditis, musculoskeletal lesions, spondylitis and neurobrucellosis) some of which are fatal if untreated. Additionally, *Brucella* spp. are the most common cause for laboratory infections and are considered a biosecurity threat due to their high infectivity.

In Morocco only few data on *Brucella* infections in humans are published. During an outbreak of human brucellosis in July 2017, the National Institute of Health in Rabat, Morocco, received 11 serum samples from a private laboratory in southern Morocco. These samples were evaluated by IgM ELISA, Rose bengal test and real-time PCR. Using ELISA and Rose Bengal test, 10 serums were diagnosed positive, while the one negative sample was confirmed as *Brucella* positive using real-time PCR.

Serology and real-time PCR detection play important roles in the diagnosis of *Brucella*. Few laboratories have the resources and expertise to perform these tests which facilitate the early diagnosis of brucellosis.

The here documented brucellosis outbreak demonstrated the need to stably implement these methods, taking into account the advanced biosafety procedures required for this highly infective pathogen. For this purpose our laboratory is cooperating with the Robert Koch Institute in the German Biosecurity Programme.
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