18th MEDICAL BIODEFENSE CONFERENCE

22 Oct - 25 Oct 2023, Munich, Germany

Medical Biodefense Conference

Honour the Past, Embrace the Future

SCIENTIFIC PROGRAM & ABSTRACTS

organized by
Bundeswehr Institute of Microbiology

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Dear Colleagues:

Welcome to the 18th Medical Biodefence Conference in Munich!

We are pleased to have more than 450 attendees from 50 nations joining us for this important event. Our programme is full of valuable insights from national and international experts in the field. You all bring a wealth of experience and expertise, which has great potential to expand our comprehension of biodefence.

Get ready for an informative and stimulating experience with engaging lectures that explore the latest research and advancements in the field. But it's not limited to lectures - get inspired by talks that push beyond the conventional. Our lineup includes poster presentations to visually showcase novel ideas and discoveries. It's an excellent chance to engage directly with other researchers and stay updated on their most recent advancements in the sector.

To round things off, for the first time we will have a panel discussion that will spark conversations about the challenges of communicating between science and policy in biosecurity research. Don't miss the opportunity to participate in a dialogue that focuses on a current issue in our field.

Thank you for being a part of this global gathering dedicated to advancing our collective knowledge in medical biodefence. Let us work together to achieve a safer and healthier future through the exchange of ideas and collaborative efforts.

Kind regards,

Prof Roman Wölfel, MD, PhD, DTMH

Colonel and Institute Director

Program at a glance (Part I)

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

Sunday, October 22

Time	Ernst-von-Bergmann barracks, Munich, Germany
15:00 | 18:00	Early Registration
18:00 | 22:00	Ice Breaker

Monday, October 23

Time	Audimax	Garden Hall	Foyer
09:00 | 10:30	A Opening Ceremony		
	Coffee Break		Poster Exhibition
11:00 | 12:30	B Medical Counter-measures - Antimicrobials and Antivirals	C A license to kill: An update on biological toxins	
	Lunch Break		
13:30 | 15:30	E Bacteriophages for Diagnostics and Therapy 1	D Outbreak Management	
	Coffee Break		
16:00 | 18:00	F Panel Discussion Biosecurity Research	G Bacteriophages for Diagnostics and Therapy 2	
19:30 | 22:00	Conference Dinner at Ernst-von-Bergmann barracks Neuherbergstraße 11, 80937 Munich, Germany		

Program at a glance (Part II)

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

Tuesday, October 24

Time	Audimax	Garden Hall	Foyer
08:30 | 10:30	H Genomics, Phylogeny and Surveillance	I Diagnostics and Detection	Poster Exhibition
	Coffee Break		
11:00 | 12:30	J Medical Counter-measures - Immunology and Vaccines	K Open Topics	
	Lunch Break		
13:30 | 15:30	L Strategies, Concepts and Analyses		Poster Session
	Coffee Break		
16:00 | 18:00	M Case Reports (TED-Session)	N German Biosecurity Programme	Poster Exhibition

Wednesday, October 25

Time	Audimax	Garden Hall	Foyer
08:30 | 10:45	O Zoonoses		
	Coffee Break		
11:00 | 12:00	P Poster Awards and Farewell		

Oral Presentations on Monday, October 23

Audimax / 09:00 ... 10:30

A Opening ceremony

Chairs: Roman Wölfel (DEU) and Joachim J. Bugert (DEU)

AO 01 ☣ 45+5min

Old agents new risks — the dirty dozen reassessed

LE Hensley
National Bio and Agro-Defense Facility, USDA Agricultural Research Service (ARS), Manhattan, KS, USA

We are living in a period of unprecedented scientific and technological advancements. These advancements are being globalized and mainstreamed at a rate never imagined. Climate change and geopolitical instability are just two examples of the complex factors impacting the risk of infectious diseases and our ability to respond to them. The average individual is estimated to come into contact with 60000 microbes a day. Only a fraction of these microbes have the potential to be harmful. Variables including the agent itself, health status of the exposed person or animal, exposure amounts, exposure routes, genetic modification of organisms, and co-infections, are all potential risk modifiers. New threats will emerge, and older threats may re-emerge as our climate, social, and agricultural practices change. Over the last 30 years, scientists have changed their approaches to studying the deadliest of the disease threats and how they respond to outbreaks of high-consequence pathogens. Technological advances have allowed scientists and clinicians to reimagine what is possible and to reduce the time to respond, control, and treat new emerging disease threats. These same advances have raised ethical and security concerns that the scientific community must continue to proactively address to maintain the public's trust. This presentation will review the evolution of research for biodefense and high-consequence pathogens and the development of response research as a critical component of our national public health and defense postures.

Audimax / 11:00 ... 12:30

B Medical Countermeasures - Antimicrobials and Antivirals

Chairs: Joachim J. Bugert (DEU) and Brett E. Swierczewski (USA)

BO 01 ☣ 12+3min

European multi-center study to establish MIC and zone diameter epidemiological cut-off (ECOFF) values for *Bacillus anthracis*

F Dematheis[1], V Manzulli[2], E Matuschek[3], D Jacob[4], M Mori[5], F Melzer[6], MC Elschner[6], A Kedrak-Jablonska[7], S Budniak[7], R Grunow[4], G Kahlmeter[3], D Galante[2], S Zange[1]
1- Bundeswehr Institute of Microbiology, Munich, DEU; 2- Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, ITA; 3- EUCAST Development Laboratory, Växjö, SWE; 4- Robert Koch Institute, Centre for Biological Threats and Special Pathogens (ZBS), Berlin, DEU; 5- Belgian institute for health, Bacterial zoonoses unit, Brussels, BEL; 6- Friedrich-Loeffler-Institute (FLI), Institute of Bacterial Infections and Zoonoses (IBIZ), Jena, DEU; 7- National Veterinary Research Institute, Puławy, POL

Background: *Bacillus anthracis*, the etiologic agent of anthrax, is a zoonotic microorganism that mostly affects herbivorous mammals, but can be transmitted to humans by contact with infected animal or their products. It is endemic almost worldwide, and it is considered a category A bioterrorism agent. If not promptly treated, *B. anthracis* infection can progress rapidly and has a high mortality rate, underpinning the importance of a timely and effective antimicrobial treatment. By now, for this microorganism, no antimicrobial susceptibility testing (AST) standards are available. Therefore, in this study, we aimed at setting up, in collaboration with EUCAST, epidemiological cut-off (ECOFF) values to distinguish between wild-type (WT) or not microorganisms.

Materials and methods: Under the framework of an EU-funded Joint action 335 *B. anthracis* isolates (17 to 146 per center) from human, environmental and animal origin were tested at 6 study sites against 10 therapy relevant antimicrobials by means of disc diffusion (DD) method and broth microdilution (BMD). Each center validated the methods testing 3 quality control (QC) strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) over 10 days and comparing the results with the EUCAST QC tables. AST was performed according to EUCAST, but with reduced incubation time compared to ISO 20776-1. *B. anthracis* MIC and DD distributions were submitted to EUCAST and aggregated results were curated in accordance with EUCAST SOP10.2.

Results: For each drug investigated, ECOFF values were defined based on 330 to 335 observations. DD and BMD data distributions, revealed a WT phenotype for the majority of the isolates. Three strains with benzylpenicillin MIC values of 32 mg/L were found, indicating resistance to this drug. MIC values above the defined ECOFF values were observed in a few strains, indicating the presence of low level resistance towards 6 antimicrobials. The genetic background to the resistance mechanisms and the phenotypic shifts observed remain to be investigated.

Conclusions: In this multi-centre study, we validated the use of BMD and DD methodologies for AST of *B. anthracis* and determined MIC and zone diameter ECOFFs for 10 antimicrobial agents. The ECOFFs can now be used to distinguish between WT and non-WT *B. anthracis*. Together with clinical data our results will pave the way for EUCAST to determine clinical MIC breakpoints for this microbial target.

BO 02 ☣ 12+3min

In vitro testing of antimicrobial agents for use against *Coxiella burnetii.*

HK Miller, GJ Kersh
Centers for Disease Control and Prevention, DVBD/RZB, Atlanta, GA, USA

Since 1999 doxycycline and hydroxychloroquine have been the standard treatment for chronic Q fever, a life-threatening disease caused by the bacterial pathogen, *Coxiella burnetii*. Alternative therapies are needed due to lengthy treatment times, drug tolerance, high mortality rate, and resistant strains. A literature search was conducted to identify studies that screened large panels of drugs against *C. burnetii* to identify novel targets for potential off-label use. Twelve candidate antimicrobials approved by the United States Food and Drug Administration were selected and minimum inhibitory concentrations (MICs) determined against a low virulence strain. Rifabutin and rifaximin were the best performing antibiotics tested with MICs of ≤ 0.01 μg mL^{-1}. These top candidates were screened alongside 2 drugs of the same class, rifampin (well-characterized), and rifapentine (never tested against *C. burnetii)*. These were screened against virulent strains of *C. burnetii* representing clinically relevant genotypes across the US. Rifapentine was the most effective across 2 strains in THP-1 cells with a MIC ≤ 0.01 μg mL^{-1}. In A-498 cells, the MIC for rifapentine was 0.01 μg mL^{-1} for 3 of the 4 strains tested; however, rifabutin and rifampin had MICs ≤ 0.01 μg mL^{-1}. Importantly, rifabutin demonstrated bactericidal activity at 0.1 μg mL^{-1}. Rifapentine and rifabutin are promising candidates for alternative Q fever treatments given their efficacy *in vitro*.

BO 03 ☣ 12+3min

Virucidal activity of essential oils

D Amatore[1], G Grilli[1], MS Lia[1], A De Domenico[1], A Amoroso[1], G Petralito[1], F Molinari[1], S Fillo[1], A Ciammaruconi[1], R De Santis[1], F Arduini[2], F Lista[1]
1- Defense Institute for Biomedical Sciences, Rome, ITA; 2- University of Rome Tor Vergata, Department of Chemical Science and Technologies, Rome, ITA

Essential oils have been traditionally used for the treatment of different diseases due to their multiple biological activities such as anti-inflammatory, antioxidant, immunomodulatory, antimicrobial. Our study is aimed at evaluating the virucidal activity of Eugenol and Carvacrol on Influenza virus H1N1, Chikungunya virus (CHIKV), Sars-Cov 2 and MS2 phage.

Virucidal activity was evaluated by TCID50 or Plaque assay. Briefly, after incubation of the viruses with the oils at final concentration of 0,5%, ten fold serial dilutions were performed and inoculated in a confluent cell monolayer for 1 hour at 37°C. Then, Flu virus was subjected to TCID50 assay, and the cytopathic effect was observed 48 hours after the infection. Sars-CoV 2 and CHIKV were subjected to Plaque assay and after 4 days plaque forming units were counted. As for MS2 phage, it was incubated with the oils and then was spotted on *E. coli*-containing agar overlay. 24 hours after the inoculum lysis areas were counted.

The preliminary results demonstrated that Carvacrol and Eugenol inhibit the enveloped viruses in a few minutes. On the contrary, MS2 shows more resistance to the action of the oils even at 60

minutes incubation. Overall, these preliminary data suggest that essential oils exert a potent virucidal effect probably by affecting viral membranes and influencing the virus ability to enter host cells. Further studies are in progress to better understand the virucidal activity of these essential oils.

BO 04 ☣ 12+3min

Determining a human clinical dose for the development of Brincidofovir for Smallpox Disease under the FDA Animal Rule

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1- Emergent BioSolutions, London, GBR; 2- Emergent BioSolutions, Gaithersburg, MD, USA; 3- Emergent BioSolutions, Winnipeg, CAN; 4- Chimerix, Durham, NC, USA

Background: Despite eradication of smallpox, concerns remain about its potential use as a bioweapon. Brincidofovir (BCV) is an FDA-approved oral lipid conjugate of cidofovir with potent *in vitro* activity against *Variola* virus, the causative agent for smallpox.

Methods: In accordance with FDA's Animal Rule, two well-characterised animal models, rabbitpox and mousepox, demonstrated clinical efficacy of BCV. These are closely related to smallpox, with similar genomic and clinical characteristics. The primary endpoint was survival. Effective human doses were modelled and simulated from therapeutic exposures to BCV in animal models. Circulating BCV and peripheral blood mononuclear cell (PBMC) concentrations of the active metabolite, cidofovir diphosphate (CDV-PP), confirmed adequate exposure over a 2-week treatment course in humans.

Results: Results showed statistically significant improved survival in the animal models in all BCV arms versus placebo, even when started beyond the midpoint of disease progression. In modelling BCV and CDV-PP maximum concentrations (C_{max}) and area under the curve (AUC) in healthy humans, 200 mg weekly provided exposures to BCV and CDV-PP in excess of efficacious levels in rabbits.

Conclusions: Efficacy studies in rabbitpox and mousepox models demonstrated improved survival with BCV versus placebo. Human dose modelling demonstrated positive ratios of C_{max} and AUC in healthy humans to those in healthy and infected rabbits. Both contributed to FDA approval of BCV.

BO 05 ☣ 12+3min

Tecovirimat for the Treatment of Human Pathogenic Poxviruses: Focus on Monkeypox

DW Grosenbach
SIGA Technologies, Poxvirus Research, Corvallis, OR, USA

On January 10, 2022, SIGA Technologies, Inc. received approval from the European Medicines Agency for the Marketing Authorisation Application of oral tecovirimat. The EMA approval includes indications for smallpox, mpox (monkeypox), cowpox, and vaccinia complications following vaccination against smallpox, which represents the major human pathogenic orthopoxviruses. Smallpox has been eradicated as a naturally occurring disease yet remains a dire threat as a biowarfare or bioterror agent. Cowpox and vaccinia usually cause mild self-resolving diseases although they may be fatal in immunocompromised hosts. Mpox (previously referred to as monkeypox) is an emerging public health threat and prior to the global outbreak in 2022 the disease was identified in 11 African countries, where it was considered endemic. Mpox had only been exported to the United Kingdom, United States, Israel, and Singapore by travelers returning from visits to West Africa. Then, in 2022, a global outbreak occurred with nearly 90,000 confirmed cases in 111 locations, resulting in 146 deaths. While less severe than smallpox, historic case fatality rates ranged from 1% to 10% depending on the virus clade but this recent outbreak was of a less virulent strain and deaths were more rare than typical. The growing number of cases in Africa and the exportation outside the continent highlight the pandemic potential of this virus. The incidence of disease is expected to increase due to continuing habitat encroachment and the increasingly immunologically naïve population no longer protected by smallpox vaccination. Until tecovirimat approval in Europe, there was no approved therapy for mpox and patients were provided supportive care only. Prophylactic vaccination was not practical under the perceived threat that existed prior to the outbreak. Live replicating vaccines such as ACAM2000 carry inherent risks that may exceed the risk of monkeypox at the current level of endemicity, but the MVA-based vaccine, Imvanex, may be suitable for pre-, and possibly, post-exposure prophylaxis as it has a much better safety profile . Tecovirimat is currently being evaluated in numerous clinical trials worldwide to determine safety and efficacy for the treatment of mpox. Stockpiling and availability of an effective antiviral, such as tecovirimat, for the treatment of mpox may reduce the likelihood of and size of future outbreaks, and in those afflicted by mpox, it may reduce disease burden and risk of mortality.

BO 06 ☣ 12+3min

cCMP and cUMP phosphodiesterases in viral infections

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1- Bundeswehr Institute of Microbiology, Munich, DEU; 2- Hannover Medical School (MHH), Institute of Pharmacology, Hanover, DEU

In bacteria, cCMP/cUMP have a key role in defense against infection with bacterial viruses. Bacteriophages encode phosphodiesterases (PDEs; 'nucleases'; Apyc1), which cleave cCMP/cUMP, counteracting this defense. We propose that PDEs are of broader biological relevance, including cCMP/cUMP cleaving PDEs of eukaryotic viruses, which may constitute new drug targets.

Garden Hall / 11:00 ... 12:25

C A license to kill: An update on biological toxins

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

CO 01 ☣ 20+5min

High-end diagnostics of botulism during the largest outbreak of iatrogenic botulism ever noted

MB Dorner[1], C Frank[2], M Skiba[1], L Wilk[1], M Steinberg[1], S Worbs[1], H Wilking[2], K Stark[2], BG Dorner[1]
1- Robert Koch Institute, Biological Toxins (ZBS 3), Berlin, DEU; 2- Robert Koch Institute, Infectious Disease Epidemiology (FG35), Berlin, DEU

Iatrogenic botulism (IB) is an adverse event after the injection of botulinum neurotoxin (BoNT) for medical or cosmetic purposes. IB is usually characterized by mild symptoms frequently involving the muscles adjacent to the injection site. The Consultant Laboratory for BoNT-producing Clostridia at the RKI was involved in the investigation of a highly unusual outbreak of travel-associated iatrogenic botulism: 30 out of 33 patients from Germany known to have received intra-gastric injection of BoNT/A for weight reduction in Istanbul developed symptoms of botulism, some of them were admitted to the ICU. The low concentration of BoNT in IB usually prevents laboratory confirmation in human serum using standard assays (e.g., mouse bioassay). Indeed, state-of-the-art diagnostics of botulism is generally challenging due to the high molecular variability within the BoNT family, its limited time window for detection in serum and the ultimate sensitivity needed. Based on a comprehensive method evaluation comparing different immunological, spectrometric and functional approaches, the detection of BoNT/A succeeded in the current IB outbreak: Applying an Endopep-MS assay and an Endopep-suspension immunoassay based on neoepitope specific monoclonal antibodies delivered positive results in 5 and 9 out of the 12 initial IB patients analyzed. The lessons-learned for botulism diagnostics in this largest outbreak of IB ever noted will be discussed.

CO 02 ☣ 12+3min

X-BAT: Research on and generation of a decavalent equine antitoxin counter-acting deliberate botulinum neurotoxin attacks

JM Modenbach[1], C Klepka[1], A Przykopanski[1], M Engelke[2], T Buchholz[2], J Jokiel[2], M Marechal[3], MA Nahori[3], S Volant[3], S Ladel[2], E Lemichez[3], A Rummel[1]
1- Hannover Medical School (MHH), Institute for Toxicology, Hanover, DEU; 2- Wirtschaftsgenossenschaft Deutscher Tierärzte eG (WDT), Garbsen, DEU; 3- Institut Pasteur, Paris, FRA

The Franco-German X-BAT consortium focuses on the research on and generation of an equine decavalent botulism antitoxin (X-BAT) counteracting deliberate attacks with all currently known botulinum neurotoxin types. By means of innovative antigen design and state-of-the-art production new routes in the generation of sufficient volumes of life saving BoNT antitoxin are explored. The consortium aims at providing a broadly available, EU-approved treatment of civilians and civil security forces upon deliberate BoNT intoxications.

The X-BAT consortium receives funding by the German *Bundesministerium für Bildung und Forschung* (MHH, WDT) and the French *Agence Nationale de la Recherche*.

CO 03 ☣ 12+3min

Five new certified reference materials (CRMs) for a better preparedness towards incidents with biological toxins

K Busschots[1], R Zeleny[1], B Kampa[2], S Worbs[2], M Skiba[2], A Puustinen[3], T Van Nieuwenhuysen[4], P Vanninen[3], C Rasetti-Escargueil[5], MA Nahori[5], E Lemichez[5], AS Mierzala[6], F Becher[6], H Volland[6],

S Simon[6], Y Nia[7], JA Hennekine[7], J Weisemann[8], N Krez[8], B Winter[8], A Rummel[8], T Bergström[9], J Näslund[9], D Jansson[9], C Müller[10], MA Avondet[10], M Wittwer[10], R Josuran[11], A Wenger[11], C Zaborosch[11], S Gerber[11], BG Dorner[2]
1- European Commission, Joint Research Centre, Geel, BEL; 2- Robert Koch Institute, Biological Toxins (ZBS 3), Berlin, DEU; 3- Finnish Institute for the Verification of the Chemical Weapons Convention (VERIFIN), Department of Chemistry, Helsinki, FIN; 4- Sciensano, Foodborne Pathogens, Brussels, BEL; 5- Institut Pasteur, Paris, FRA; 6- CEA-Saclay, Laboratoire d'études et de recherches en immunoanalyse, Gif-sur-Yvette, FRA; 7- ANSES, Food Safety Laboratory, Maisons-Alfort, FRA; 8- toxologics UG, Hanover, DEU; 9- Swedish Defence Research Agency, Umeå, SWE; 10- Bundesamt für Bevölkerungsschutz, Labor Spiez, Spiez, CHE; 11- Zürcher Hochschule für Angewandte Wissenschaften, Life Sciences und Facility Management, Wädenswil, CHE

Recent malicious events in Germany and France have shown the need for a better preparedness towards biotoxin incidents in Europe. One main task in the Horizon 2020 EuroBioTox project was the development of certified reference materials (CRMs). CRMs are important tools for laboratories to develop and safeguard methods for reliable biotoxin analysis. Five CRMs were produced, which are solutions of pure protein toxins in an aqueous buffer: *Staphylococcus aureus* enterotoxin B, *Clostridium botulinum* neurotoxins A1 and B1 (produced recombinantly), as well as ricin from *Ricinus communis* and abrin from *Abrus precatorius* (native proteins purified from seeds). An extensive purity and identity assessment of the materials was performed with a variety of methods (e.g. ELISA, mass spectrometry techniques, capillary gel electrophoresis, functional assays). Suitable homogeneity as well as transport and storage stability was demonstrated. Characterization measurements (e.g. using UV spectrophotometry and mouse bioassay) allowed assigning reference values to the materials. The intended use of the materials includes method calibration and performance verification. The availability of these CRMs will lead to a better comparability of results among laboratories, will allow establishing metrological traceability of results to an internationally recognized reference (e.g. International System of Unit, SI) and thus contribute to a better preparedness towards biotoxin incidents.

Reference: https://www.eurobiotox.eu

CO 04 ☣ 12+3min

Autophagic degradation is involved in cell protection against ricin toxin

Y Wu[1], C Taisne[2], N Mahtal[3], A Forrester[4], M Lussignol[2], JC Cintrat[5], A Esclatine[2], J Barbier[3], D Gillet[3]
1- University of Science and Technology of China, Institute of Immunology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, Hefei, Anhui, CHN; 2- Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, FRA; 3- Université Paris-Saclay, CEA, INRAE, Médicaments et Technologies pour la Santé (DMTS), SIMoS, Gif-sur-Yvette, FRA; 4- University of Namur, Research Unit of Biochemistry and Cell Biology (URBC), Namur Research Institute for Life Sciences (NARILIS), Namur, BEL; 5- Université Paris-Saclay, CEA, CNRS, Médicaments et Technologies pour la Santé (DMTS), SCBM, Gif-sur-Yvette, FRA

Autophagy is a complex and highly regulated degradative process, which acts as a survival pathway in response to cellular stress, starvation or pathogen infection. Ricin-induced apoptosis has been extensively studied, however, whether its intoxication via protein synthesis inhibition affects autophagy is not yet resolved. In this work, we demonstrated that ricin intoxication is accompanied by its own autophagcic degradation in mammalian cells. Autophagy deficiency, by knocking down ATG5, attenuates ricin degradation thus aggravating ricin-induced cytotoxicity. Additionally, the autophagy inducer SMER28 (Small Molecule Enhancer of Rapamycin 28) partially protects cells against ricin cytotoxicity, an effect not observed in autophagy-deficient cells. These results demonstrate that autophagic degradation acts as a survival response of cells against ricin intoxication. This suggests that stimulation of autophagic degradation may be a strategy to counteract ricin intoxication.

CO 05 ☣ 12+3min

In vivo efficacy of a polyclonal ovine antibody Fab fragment against ricin toxicity

ZA Bascal[1], A Griffiths[1], V Postila[2], C Hill[3]
1- Protherics UK Ltd, Llandysul, GBR; 2- SERB Pharmaceuticals, Frankfurt, DEU; 3- BTG International Inc, West Conshohocken, PA, USA

Introduction: Ricin is a toxin isolated from seeds of *Ricinus communis*. It has been considered a threat as a weapon due to its ease of manufacture and toxicity. There is no specific therapy for ricin intoxication. A polyclonal ovine antibody Fab (PR022) was developed by immunizing sheep with a *R. communis*-derived protein.

Objective: To establish if intravenous (IV) administration of PR022 is effective in a mouse ricin intraperitoneal (IP) model.

Methods: An LD_{50} ricin murine model was established following IP administration of ricin and confirmed in BALB/c mice. Based on the model, on Day 0 animals were administered increasing doses of the PR022 IV, 2hrs after ricin IP challenge of 6 LD_{50}. Animals were then observed for up to 14 days for survival, weighed daily and observed clinically twice daily.

Results: Ricin alone exposed mice died in <28hrs. PR022 improved survival and body weight in a dose-dependent manner. In addition, there was decreased incidence and severity of toxic signs in PR022 treated groups.

Conclusion: PR022 demonstrated in a dose-dependent manner improved survival rates and body weight following ricin challenge. PR022 has potential as a medical countermeasure to ricin toxicity.

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

D

Outbreak Management

Chairs: Katharina Müller (DEU) and Kai Kehe (DEU)

DO 01 ☣ 25+5min

UK cases of Crimean-Congo haemorrhagic fever (CCHF) and the WHO Research & Development Blueprint Roadmap for CCHF

AE Semper, TJG Brooks
UK Health Security Agency, Rare and Imported Pathogens Laboratory, Porton Down, GBR

CCHF has the widest distribution globally of the viral haemorrhagic fevers (VHF), endemic in countries from South Africa, Eastern Asia and across Eastern Europe to Spain. Cases occur seasonally every year. Humans are infected either by the bite of a tick or from contact with blood of an infected animal. The disease can be transmitted from human to human and clinically ranges from asymptomatic to overt VHF. Imported cases are rare, but we will describe 2 cases with different presentations and outcomes.

WHO is preparing a set of Research & Development Blueprint Roadmaps for epidemic prone diseases to focus efforts on disease control, especially diagnostics, therapeutics and vaccines, supported by studies on pathogenesis and surveillance. The Roadmap for CCHF is jointly led by UKHSA and WHO, supported by a Task Force of 19 International experts from affected countries and expert researchers and will be published on the WHO website for consultation. Domestic animals such as cattle, sheep and goats are an important part of the CCHF virus' natural cycle and the Roadmap therefore addresses this aspect of the disease and how it affects the intervention programmes, including the prospect of introducing anti-tick vaccines. A consultation with stakeholders — academics, healthcare officials, funders and industry — will be held in the late fall of 2023.

The presentation will discuss the roadmap and its challenges and implementation in detail.

DO 02 ☣ 12+3min

MediLabSecure, a One-Health network for the prevention of vector-borne diseases emergence at the European doors

G Mikaty[1], E Pérez-Ramírez[2], J Fernández-Pinero[2], MÁ Jiménez-Clavero[2], V Robert[3], MG Dente[4], S Declich[4], G Hendrickx[5], V Lagal[1], M Seguy[1], JC Manuguerra[1]
1- Institut Pasteur, Paris, FRA; 2- Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Valdeolmos, ESP; 3- IRD, MIVEGEC, Montpellier, FRA; 4- Istituto Superiore di Sanità, Rome, ITA; 5- Avia-GIS, Zoersel, BEL

MediLabSecure (MLS) is a capacity-building project funded by the European Commission and implemented by European organisations for the benefit of 22 EU neighbouring countries in the Mediterranean, Black Sea, and Sahel regions. MLS gathers 114 multidisciplinary laboratories and health institutions to prevent and control vector-borne diseases. MLS sponsors the integration of One Health in preparedness, surveillance, and response against zoonotic arboviruses. Experts in human and animal virology, medical entomology, public and animal health, and geospatial modelling collaborate to provide training and guidance to the beneficiaries to implement One-Health, identify pathogens and their vectors, or conduct operational studies.

MLS main goal is preparedness to respond to emerging viruses and outbreaks. Nevertheless, since 2014, numerous outbreaks and epidemics hit the region and the project adapted from preparedness to response. The epidemic of Zika, the Pandemic of

COVID-19, an outbreak of RVF in Mauritania in 2020, or more recently the outbreak of mPox, were opportunities for the network to activate. During these events, MLS supported the rapid and efficient implementation of diagnostic tools, the implementation of sequencing capacities, and numerous scientific and technical support activities.

Over the years, MLS proved to be an efficient and successful capacity-building project and created a strong One-health network of experts in the Mediterranean, black sea, and Sahel regions.

DO 03 ☣ 12+3min
❯ NATO MilMedCoE's Near Real Time Surveillance - Evaluation of the KFOR Pilot

S Ruhl[1], R Lindfield[1], M Toth[2]
1- NATO MilMedCoE, Force Health Protection Branch, Munich, DEU; 2- NATO MilMedCoE, International Relations, Budapest, HUN

NATO has a requirement for a near real time surveillance tool to identify disease outbreaks at the earliest opportunity to allow a prompt response, but no deployable tool has been available. The MILMED COE developed a NRTS tool and piloted it on the NATO Mission in Kosovo.

Ten medical treatment facilities in KFOR piloted the tool which consisted of an app on a smart device. All patients presenting with a new condition were entered into the app by a clinician. If the patient's main presenting symptoms mapped to one or more of 40 symptoms on the app then their symptoms were entered. No patient identifiable information was collected. Each MTF and the Medical HQ staff (JMED) had access to an analysis tool which provided a summary of the data collected. Specific combinations of symptoms resulted in an alert warning email to the JMED and MTF.

An evaluation was conducted at two time points and consisted of data analysis and semi-qualitative interviews with key informants from each participating MTF and JMED team.

1351 new patient reports were entered into the app during the pilot. 851 reports had symptoms recorded. The leading symptoms were pain-in-throat, cough and temperature. Two investigations were launched based on reporting in the app; a cluster of skin lesions and two cases of diarrhoea. No significant outbreaks were reported to the MILMED COE during the pilot or picked up by the app.

The app was easy to use and the symptom list was described as being comprehensive. The analysis tool was not viewed or used regularly by MTFs however the JMED team used it frequently. Alerts were considered extremely important by both the MTFs and JMED team.

The additional requirement to collect EpiNATO-2 syndromic surveillance as well as NRTS during the pilot was viewed as an issue and it was felt that the app would be an appropriate and simple way to report EpiNATO-2.

The KFOR pilot provided an excellent opportunity to pilot the tool in a NATO Mission and established that the process of collecting data and reporting issues worked well. Further validation and analysis of the tool is required before it can be rolled out further.

DO 04 ☣ 12+3min
❯ Hospital Ship Ventilation Study

SC Francesconi
Defense Threat Reduction Agency, RD CBA, Fort Belvoir, VA, USA

The United States have two hospital ships: converted oil tankers modified to contain beds for 1,000 patients. Built in the 1970's, they were designed to provide medical center care for battlefield trauma wounds. They are currently used for humanitarian outreach: delivering food and medical supplies after tsunamis, earthquakes, and other environmental disasters worldwide. Both were used during the recent pandemic to reduce hospital overcrowding in New York City and Los Angeles. Despite having HEPA filtered air in the twelve surgical suites, the wards that house the patients have far fewer air changes per hour (ACH)—never an issue until a highly infectious respiratory virus appeared.

We will present video illustrating the placement of portable HEPA filters to remove a simulated pathogen as efficiently as possible. To measure this efficiency, we used sixty laser induced fluorescence particle counters arrayed within and in neighboring compartments. By moving the HEPA filters within the space and repeating the exposure, we can determine the optimum placement for particle removal. The take home lesson is knowing when to apply the HEPA filters, as running them all day and all night is not only unnecessary when no pathogens are present, but expensive as it will clog the filters quicker. For this reason, we are developing a miniaturized aerosol sensor that can turn the filter units on and off when needed.

DO 05 ☣ 12+3min
❯ Identification of the 2022 Mpox outbreak: the impact of the UK Imported Fever Service

N Ayton[1], H Callaby[2], C Houlihan[1], J Osborne[2], T Rampling[1], NC Gordon[2]
1- UKHSA, RIPL, London, GBR; 2- UKHSA,

RIPL, Salisbury, GBR

Background: The Imported Fever Service (IFS) is a unique clinical helpline run by the UKHSA Rare and Imported Pathogens Laboratory (RIPL), in conjunction with the Hospital for Tropical Diseases and the Liverpool School for Tropical Medicine. The IFS provides a 24/7 clinical helpline for advice on risk assessment, management, testing and infection control for viral haemorrhagic fevers and other high consequence infections, as well as general advice on tropical or rare bacterial and viral infections.

Method: A case of suspected Mpox was notified to the IFS on 6 May 2022, in a patient recently returned from Nigeria. Following this initial case, 2 further, unrelated clusters were identified and the subsequent notification process led to the recognition of the global Mpox outbreak which, to date, has resulted in over 80,000 confirmed cases worldwide.

Over the following days, the IFS received a significant surge in calls from clinicians requesting advice on Mpox risk assessment, diagnosis and management. Consequently, a dedicated UK-wide Mpox helpline was set up to support clinicians dealing with suspected cases, and to ensure timely reporting of results. In parallel, the RIPL laboratory developed a dedicated 7-day Mpox testing service, with same day testing of samples arriving before 9am and results phoned the same evening to referring clinicians by the Mpox helpline staff. All positive cases were centrally notified to public health teams and to the national High Consequence Infectious Disease network, who coordinated admissions and drug treatment for patients with severe disease.

Results: Over 500 calls were made to the IFS Mpox helpline. The dedicated phoneline allowed rapid recognition of transmission risk factors. The rapid testing service minimised diagnostic delays and allowed earlier instigation of public health measures such as contact tracing, whilst allowing patients who tested negative to deisolate as early as possible.

Conclusion: A single source for advice and testing, via the IFS, was key in identifying the Mpox outbreak in 2022. Providing a dedicated phoneline and streamlined testing / resulting service supported the public health response allowing early identification of contacts. This also enabled rapid referral of patients with severe disease to the national expert network. The Mpox outbreak demonstrated the utility of the IFS model in emerging outbreaks, including outbreaks involving potential biothreat agents.

DO 06 ☣ 12+3min

❯ Lessons from COVID: Establishment of QIAGENs pandemic prepardness initiative

S Reister[1], M Destito[2], D Lueerssen[3], A Blacha[4], S Johnson[5], K Te Kaat[1]
1- QIAGEN, Product development MDx, Hilden, DEU; 2- QIAGEN, Medical Affairs, Hombrechtikon, CHE; 3- QIAGEN, Product development MDx, Barcelona, ESP; 4- QIAGEN, Medical Affairs, Post Market surveillance, Manchester, GBR; 5- QIAGEN, Clinical Affairs, Post Market surveillance, Manchester, GBR

The COVID-19 pandemic hit all diagnostic companies and all public health infrastructure like a hailstorm. Immediate fast reaction was rolled out, but still needed some time to adapt and to ramp up production capacities. Furthermore, it took time to identify the new agent. The first registered case was December 8^{th} 2019, but evidence is available that the virus was previously circulating.

With the first full genome sequences available, targeted assays were developed and led ultimately in less than a month to the first integrated syndromic panel (QIAstat SARS-CoV-2 Panel). This was only possible due to the Cartridge configuration of the non-COVID Respiratory Panel having an unused reaction channel to facilitate fast adaptability.

With this first step done the next challenges arose, namely fast mutation of the virus requiring a sophisticated Post Market surveillance tool which was fed until now with more than 10.000.000 full genome sequences to check for putative critical mutations which would lead to false negative results.

Beside the operational and technical preparedness QIAGEN has initiated a global surveillance system checking for local reports covering disease outbreaks as well as a Triage system to react on these. As example Mpox was identified, classified as actionable and the QIAstat Viral vesicular panel (RUO) was launched within 7 weeks as first syndromic panel.

DO 07 ☣ 12+3min

❯ The Tunisian mobile laboratory: a national strategic tool for health support

M Ben Moussa
Military Hospital Tunis, Department of Virology, Tunis, TUN

During health crises and biological threats; it is essential to have rapid and effective support tools, essentially in the field, as demonstrated by the last SARS-CoV-2 pandemic where the diagnostic component was lacking in certain countries and regions. Among these support tools; the Tunisian mobile laboratory for microbiological diagnosis had played an essential role in screening and health support in the field. This system is endowed with

flexibility, speed of deployment, enormous molecular diagnostic techniques and competent human resources. This allowed him to play a big role during COVID19 on the Tunisian national level and to support certain events and activities. Thanks to its quality; he is able to support health teams in human or veterinary medicine during crises, mainly in the field. Its enormous diagnostic qualities have enabled it to be considered as an essential strategic tool in the Tunisian health system in the context of the management of biological threats and risks.

Audimax / 13:30 ... 15:30

E

Bacteriophages for Diagnostics and Therapy 1

Chairs: Peter Braun (DEU) and Martin J. Loessner (CHE)

EO 01 ☣ 20+5min

Engineering Phage Tail Fibers and Reporter Phages for Rapid Pathogen Detection and Patient Selection for Phage Therapy

M Dunne
ETH Zurich, Institute of Food, Nutrition and Health (IFNH), Zurich, CHE

Fast and reliable detection of bacterial pathogens in clinical samples, contaminated food products, and water supplies is crucial for improving clinical outcomes and reducing the socio-economic impact of diseases. Bacteriophages (phages), as natural predators of bacteria, exhibit unparalleled specificity in binding to their hosts and rapidly delivering and replicating their viral genome. Leveraging these unique attributes, phages, and phage-encoded proteins such as tail fibers, tail spikes, and endolysins, have been harnessed to develop a diverse range of diagnostic assays, surpassing conventional culture-based and molecular detection methods.

As examples of our research, we have capitalized on the inherent specificity of the *Salmonella* phage S16 long tail fiber (LTF) to create an innovative enzyme linked LTF assay (ELLTA). This assay combines LTF-coated magnetic beads with horseradish peroxidase-conjugated LTF, facilitating quick and sensitive detection of this critical foodborne pathogen. Additionally, by conjugating tail fibers onto latex beads, we developed an agglutination-based assay for detecting *Burkholderia pseudomallei*, the causative agent of melioidosis in endemic regions of Thailand and Southeast Asia.

Highly specific and rapid pathogen detection can be achieved through bacteriophage-mediated delivery and host-dependent expression of luciferase reporter genes, a diagnostic approach known as 'reporter phage'. Using this approach, we engineered six distinct nanoluciferase reporter phages and devised a urinalysis assay capable of detecting and differentiating the three predominant UTI pathogens (*E. coli*, *Klebsiella* spp., and *E. faecalis*) with high specificity (97%, 98%, 98%) and sensitivity (66%, 81%, 81%) at a resolution of $\geq 10^3$ CFU/mL. This phage-based diagnostic platform shows promising potential for prompt bacterial UTI diagnosis in point-of-care settings and has been further developed into a companion diagnostic to determine phage viability and patient eligibility for phage therapy using phages engineered to express and release heterologous effectors such as bacterocins or endolysins.

EO 02 ☣ 20+5min

Recombinant receptor binding proteins of bacteriophages as versatile tools for detection of highly pathogenic bacteria

L Reetz[1], S Suppmann[1], G Grass[2], P Braun[1]
1- Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Immunology, Infection and Pandemic Research IIP, Penzberg, DEU; 2- Bundeswehr Institute of Microbiology, Munich, DEU

For highly pathogenic bacteria, such as *Bacillus anthracis*, *Yersina pestis*, *Burkholderia pseudomallei* and *Brucella* spp., rapid and unambiguous detection is crucial for timely antibiotic therapy of infected patients. While polymerase chain reaction (PCR) is the gold standard for diagnostics of most infectious diseases, antibody-based assays that detect specific antigens of the pathogen are commonly used as confirmatory methods. However, these antibodies often feature insufficient specificity due to the high degree of relatedness of these pathogens to their non- or less pathogenic relatives. Receptor binding proteins (RBPs) of bacteriophages, which mediate recognition and binding to the host cell, represent a promising alternative to antibodies. Here, we identified RBPs of a variety of phages specific for *Bacillus anthracis*, *Yersina pestis*, *Burkholderia pseudomallei* and *Brucella* spp. and utilized them to develop a set of novel tools for detection of these

notorious pathogens. For this, recombinant RBPs were produced as fusions with different reporter proteins, such as fluorescent proteins or enzymes. In addition, RBPs were coupled to magnetic beads to serve as highly specific capture molecules for bacterial pathogen enrichment or isolation approaches.

EO 03 ☣ 15+5min

Rapid detection of antibiotic resistance and phage-associated lysis of bacteria by single-cell Raman technology

HK Yosef[1], JJ Bugert[2], JA Hammerl[3]
1- microphotonX GmbH, Application/Lab, Tutzing, DEU; 2- Bundeswehr Institute of Microbiology, Munich, DEU; 3- German Federal Institute for Risk Assessment, Biological Safety, Berlin, DEU

Background: The dramatic increase in mortality associated with antimicrobial resistance (AMR) has emphasized the need for faster diagnostic tools and alternative therapeutic strategies. Bacteriophage has emerged as an alternative therapy to overcome AMR, leading to the direct lysis of pathogenic bacteria. However, such a therapeutic strategy also requires a fast evaluation method to define the suitable phage for each infection.

Methods: The combination of Micro-Raman spectroscopy and laser trapping is an attractive technology for the rapid identification of bacteria, susceptibility testing, and phage evaluation. Laser trapping creates a force field that attracts and immobilizes single bacteria in the laser focus, while Raman spectroscopy records the molecular information simultaneously.

Results: 120 clinical isolates of different *Klebsiella* species were investigated, of which 80 were classified as multidrug-resistant (MDR). The isolates were measured using a micro-Raman platform (BiospexmicroPhotonX GmbH) and analysed using linear discriminant analysis. A classification accuracy of 95% was reached in distinguishing MDR *Klebsiella pneumoniae* (Kp) from other susceptible *Klebsiella* species. Furthermore, two Kp isolates were treated with phages lytic for the isolates and other phages unable to produce plaques on them. Raman analysis revealed distinctive molecular dynamics associated only with the phage lytic effect on Kp.

Conclusion: Raman technology provides a fast and accurate solution for the detection of MDR and antimicrobial/phage susceptibility testing.

EO 04 ☣ 15+5min

Rapid Detection and Real-Time Antibiotic Susceptibility Testing of *Klebsiella pneumoniae* and *Yersinia pestis* Using Recombinant Reporter Phages

S Kachel[1], S Braun[1], JJ Bugert[2], P Braun[1]
1- Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Immunology, Infection and Pandemic Research IIP, Penzberg, DEU; 2- Bundeswehr Institute of Microbiology, Munich, DEU

Infections caused by highly pathogenic bacteria like *Yersinia pestis* are rare, however, the pathogens still pose a major biosecurity risk due to the potential misuse for biological warfare or bioterrorism. In contrast, the massive emergence of multi-drug-resistant (MDR) bacteria, such as *Klebsiella pneumoniae*, constitutes an enormous threat to global health as MDR-associated treatment failure causes high mortality rates in nosocomial infections. In both cases, rapid pathogen detection and antibiotic resistance screening are crucial for successful therapy and thus patient survival. Reporter phage-based diagnostics offer a way to speed up pathogen identification and resistance testing as integration of reporter genes into highly specific phages allows real-time detection of phage replication and thus living host cells. Here, we developed and engineered highly specific reporter phages which produce nanoluciferase (nLuc) upon host infection and thus enable rapid detection of *K. pneumoniae* and *Y. pestis* cells in clinical matrices within a few hours. At the same time, these reporter phage assays allow for real-time antibiotic susceptibility testing and therefore rapid identification of suitable treatment options.

EO 05 ☣ 25+5min

Novel tools from bacteriophage: tiny killers and live savers

MJ Loessner
ETH Zurich, Institute of Food, Nutrition and Health (IFNH), Zurich, CHE

Antibiotic resistance represents a great challenge for health care worldwide, and novel strategies to control infection by drug-resistant bacteria are urgently needed. While the natural enemies of bacteria, bacteriophages, offer great host specificity and killing activity, their therapeutic potential is naturally limited by narrow host-ranges, insufficient antimicrobial activity, lysogeny, and rapid emergence of resistance. However, synthetic biology and genetic engineering of phage genomes can overcome these limitations and offers new possibilities for the design of smart and effective phage-based antimicrobials.

We use a synthetic phage engineering approach, based on in-vitro DNA assembly and subsequent reactivation (rebooting) of synthetic phage genomes within suitable host cells. To enable efficient rebooting of phage genomes in Gram-positive bacteria,

we developed a bacterial L-form based platform, providing less stringent surrogate hosts for phage amplification. To enhance recombination-based engineering of very large phage genomes unsuitable for synthetic fragment assembly, CRISPR-Cas based counterselection systems were established in various phage hosts. Using these platforms, we (i) converted temperate phages to virulent ones, (ii) produced phages carrying a broad variety of additional payload genes for expression in the infected host, (iii) created phages showing extended killing of completely unrelated bacteria by phage-encoded cross-acting antimicrobials, (iv) designed transducing but non-replicating killer phage, and (v) broadened phage host ranges by structure-guided design of receptor binding proteins.

Besides using bacteriophages, another promising and successful approach is to harness the high specificity and strong enzymatic lysis activity of phage-encoded peptidoglycan hydrolases, the endolysins. Here, we have made significant progress by not only optimizing enzyme activity and in vivo half-life by domain shuffling and fusion to non-phage sequence, but also targeted modification of the enzymes for fine-tuned application in serum and blood, tissue, and even intracellular environments.

Audimax / 16:00 ... 18:00

F Biosecurity Research - Challenges in Communication between Science and Politics (Panel Discussion)

Moderator: Katharina Müller (DEU)

Panelists:

Silke Bellmann
Federal Foreign Office, Berlin, DEU

Julia Burr
Institute for Defense Analyses, Alexandria, VA, USA

Petra Dickmann
dickmann risk communications drc/, London, GBR

Una Jakob
Peace Research Institute, Frankfurt am Main, DEU

Roman Wölfel
Bundeswehr Institute of Microbiology, Munich, DEU

Garden Hall / 16:00 ... 17:00

G Bacteriophages for Diagnostics and Therapy 2

Chairs: Joachim J. Bugert (DEU) and Zuzanna Drulis-Kawa (POL)

GO 01 ☣ 25+5min

Biology-based machine learning to predict phage-host specificity for the clinical phage therapist

D Boeckaerts[1], C Pas[1], A Latka[2], C Ferriol González[3], R Sanjuan[3], P Domingo-Calap[3], L Fieseler[4], M Stock[1], B De Baets[1], Y Briers[1]
1- Ghent University, Ghent, BEL; 2- Ghent University, University of Wroclaw, Ghent, BEL; 3- Universitat de Valencia-CSIC, Valencia, ESP; 4- ZHAW School of Life Sciences and Facility Management, Wädenswil, CHE

Introduction: Artificial intelligence has been instrumental in driving disruptive advancements in life sciences. A main remaining limitation in the context of phage therapy is the computational prediction of phage-host interactions at the strain level. To address this limitation, we previously proposed

a biology-informed multilayer machine learning approach for elucidating phage-host interactions at the strain instead of the genus or species level, providing a practical tool for phage therapy.

Objective: We introduce the first layer of this approach, named PhageHostLearn, leveraging knowledge on the initial interaction between phage receptor-binding proteins (RBPs) and bacterial surface receptors. As such we aim to push predictions to the strain level, a key requirement for the clinical phage therapist.

Methods: In recent years, we have gained a deep understanding of the modularity and horizontal evolution of phage RBPs, validated by bio-informatic analyses, protein engineering and phage genome engineering for both *Escherichia coli* and *Klebsiella pneumoniae*. We blend these insights with a large experimentally validated training set to accurately predict the interactions between RBPs and bacterial receptors for *Klebsiella* phage-bacteria pairs at the strain level.

Results: We have rigorously evaluated PhageHostLearn through comprehensive *in silico* and *in vitro* experiments, focusing on its practical implementation in identifying matching phages for a bacterial strain emerging in a clinically relevant setting. Furthermore, we have made PhageHostLearn publicly available as a tool that can be continually enhanced and refined.

Conclusions: The PhageHostLearn system generates actionable predictions for the clinical phage therapist who is selecting the right phage for the patient.

GO 02 ☣ 12+3min

Cell-free production of personalized therapeutic phages targeting multidrug-resistant bacteria

K Vogele[1], S von Schönberg[2], JJ Bugert[3], P Großmann[1], GG Westmeyer[4]
1- Invitris GmbH, Munich, DEU; 2- Physics of Synthetic Biological Systems E14, Physics Department and ZNN, TUM, Munich, DEU; 3- Bundeswehr Institute of Microbiology, Munich, DEU; 4- Institute for Synthetic Biomedicine, Bioscience, Munich, DEU

Bacteriophages are a promising therapeutic approach to combat rapidly increasing numbers of infections with multidrug-resistant (MDR) bacteria. However, the broad implementation of bacteriophage therapy is currently impeded by a lack of safe production standards and insufficient phage characterization. We utilized a cell-free expression system to produce high titers of bacteriophages, fully omitting the use of living bacteria.

This synthetic system is able to perform all steps of phage replication, which we show by use of different technologies: phage DNA is replicated (shown by qPCR), phage proteins are expressed from the DNA template, which we show using mass spectrometry, and finally expressed phage proteins self-assemble into fully functional phages as verified by plaque assay.

We further developed this system into a host-independent platform for the production of phages against both gram-positive and gram-negative bacteria. At a microliter-scale, our . e We expect our cell-free methodology to enable and accelerate safe and tailor-made phage therapies against the growing number of pathogenic MDR bacteria. E. coli derived cell-free expression system produces effective doses of phages against E. coli, including enteroaggregative E. coli (EAEC), K. pneumonieae, Pseudomonas sp. and Y. pestis. By co-expressing suitable host factors, we were furthermore able to extend the range of our platform to include phages of gram-positive bacteria like B. subtilis. The use of our cell-free system for phage production in a clinical setting offers the advantage of significant reduction of impurities such as endotoxin and prophage contaminations, compared to a bacteria-based approach.

Finally, we showcase a pipeline for personalized phage therapy of a multidrug-resistant ESKAPE pathogen from isolation to in vitro production of a phage against a clinical isolate of K. pneumonia.

GO 03 ☣ 12+3min

K-sensitization strategy to improve existing treatment protocols for effective therapy against AMR *K. pneumoniae*

Z Drulis-Kawa, G Majkowska-Skrobek, T Olszak
University of Wroclaw, Department of Pathogen Biology and Immunology, Wroclaw, POL

Klebsiella pneumoniae is a WHO priority 1 pathogen displaying pan-resistance against prevalent antibiotics. Moreover, encapsulation serves as the key virulence factor protecting this bacterium against the human immune system response.

JPIAMR KLEOPATRA project proposes the use of bacteriophages and their capsule depolymerases as sustainable combinations with antibiotics. We introduce the concept of K-sensitization, which utilizes phages and their capsule-degrading enzymes to drive bacteria toward capsule depletion.

The application of capsule targeting phages leads to the selection of capsule-less/modified bacterial population accompanied by reduced resistance to the host complement system and more prone to engulfed and neutralization by phagocytic cells. Moreover, it

renders bacteria susceptible to capsule-independent phages that recognize receptors previously not accessible because of the capsule's physical barrier. It means the most effective phage cocktails against *K. pneumoniae* should be composed of capsule-dependent and -independent phages.

The K-sensitization concept provides also new diagnostic tools for encapsulated bacteria and improves the design of effective cocktails for compassionate/magistral phage therapy.

Oral Presentations on Tuesday, October 24

Audimax / 08:30 ... 10:30

H Genomics, Phylogeny and Surveillance

Chairs: Mathias C. Walter (DEU) and Andreas Sjödin (SWE)

HO 01 ☣ 12+3min

Mapping the Invisible: Global Tracking of pathogen *Francisella* in Public Metagenomic Datasets

A Sjödin
Swedish Defence Research Agency - FOI, CBRN Defence and Security, Umeå, SWE

The emergence of big data and bioinformatics has brought about a revolutionary change in our ability to monitor and track the dissemination of pathogens. In this comprehensive investigation, we delve into the worldwide distribution of *Francisella*, a bacterial genus known to pose significant biodefense challenges. Leveraging the capabilities of sourmash, a cutting-edge tool designed to index and search extensive genomic databases, we conducted a meticulous examination of all publicly accessible metagenomic datasets to detect the presence of *Francisella* sequences. This systematic search enabled us to pinpoint and retrieve pertinent datasets, which were subsequently reconstructed into Metagenomic Assembled Genomes (MAGs). These MAGs served as intricate maps, illuminating the geographic expansion and habitat preferences of distinct *Francisella* species across diverse regions of the globe.

Our study findings provide professionals with invaluable insights into the behavior of these bacteria, thereby contributing to the enhancement of surveillance practices and the implementation of more efficient management strategies for potential outbreaks. By highlighting the transformative potential of big data and bioinformatics in the field of public health and biodefense, this research paves the way for a proactive and data-driven approach to the anticipation and control of public health crises. We firmly believe that the application of innovative digital tools in this manner will play a critical role in shaping the future landscape of global health, facilitating a shift from reactive measures to proactive strategies in the face of potential disease outbreaks. Such an approach holds the promise of better preparedness, quicker responses, and ultimately, improved outcomes in safeguarding public health worldwide

HO 02 ☣ 12+3min

Novel threats - State of the art of synthetic biology

TB Johansen[1], EH Madslien[1], M Forsman[2], F Ekström[2], EM Fykse[3], S De Keersmaecker[4], N Roosens[4], K Vanneste[4], MA Fraiture[4], B Pályi[5], M Knepr Segina[6], L Zmak[6], I Tabain[6], H Jankovic[6], J Jones[7]
1- Norwegian Institute of Public Health, Oslo, NOR; 2- Swedish Defence Research Agency, Umeå, SWE; 3- Norwegian Defence Research Establishment, Lillestrøm, NOR; 4- Sciensano, Brussels, BEL; 5- National Public Health Centre, Budapest, HUN; 6- Croatian Institute of Public Health, Zagreb, HRV; 7- UK Health Security Agency, Salisbury, GBR

Introduction: The EU-project Joint Action TERROR aims to address gaps in health preparedness and cross-sectoral work in response to biological and chemical attacks. Synthetic biology is a rapidly developing field which could be misused to construct and weaponize novel biological agents. We performed a literature review to describe state of the art knowledge on dual-use potential of synthetic biology.

Methods: A systematic review was conducted for the period January 2016 to February 2022 following PRISMA guidelines. We limited our search to map and describe novel technologies that can be misused to: 1) re-create known pathogenic viruses and bacteria, 2) make existing bacteria and viruses more pathogenic and 3) make harmful biochemicals via *in situ* synthesis.

Results: A total of 1505 articles were identified, of which 1254 were excluded. Findings were summarized in five categories: 1) DNA/ RNA sequencing, 2) gene synthesis/ editing, 3) heterologous expression; 4) metabolic engineering; 5) general synthetic biology and accessibility concerns.

Discussion and conclusion: With increasing sophistication and availability of synthetic biology comes a range of potential risks. Machine learning and artificial intelligence increases the possibilities for dual-use. The accelerating development raises concern that established biosecurity measures will not be sufficient to prevent malicious use of synthetic

biology. It is essential to be prepared by having well-developed healthcare systems, efficient diagnostic tools, and a strong foundation of basic research in relevant areas.

HO 03 ☣ 12+3min

A multiplexed, high-throughput, targeted amplicon sequencing assay for biothreat detection

SL Sholes[1], S Harrison[1], C Bell[1], R Player[2], K Verratti[1], B Necciai[3], S Sozhamannan[4]
1- Johns Hopkins University, Applied Physics Laboratory, Laurel, MD, USA; 2- Datirium, LLC, Cincinnati, OH, USA; 3- Joint Program Executive Office for CBRN Defense (JPEO-CBRND), Frederick, MD, USA; 4- Joint Research and Development, Inc., Stafford, VA, USA

The gold standard in nucleic acid-based biodetection continues to be polymerase chain reaction (PCR), and many real-time PCR assays targeting biodefense pathogens for biosurveillance are in widespread use. These assays are predominantly singleplex; i.e., one assay tests for the presence of one target, found in a single organism, one sample at a time. Due to the intrinsic limitations of such tests, there exists a critical need for high-throughput multiplex assays to reduce the time and cost incurred when screening multiple targets, in multiple pathogens, and in multiple samples. Such assays allow users to make an actionable call while maximizing the utility of the small volumes of test samples. Unfortunately, current multiplex real-time PCR assays are limited in the number of targets that can be probed simultaneously due to the availability of fluorescence channels in real-time PCR instruments. To address this gap, we developed a pipeline in which the amplicons produced by a 23-plex end-point PCR assay using spiked samples were subsequently sequenced using Oxford Nanopore technology (ONT). We used DNA barcodes to sequence multiple samples simultaneously, leading to the generation and real-time analysis of sequence data using a software tool we developed called ONT-DART (ONT-Detection of Amplicons in Real Time). Overall, LoDs determined from the first 10 min of sequencing data were at least one to two orders of magnitude lower than real-time PCR. Given enough time, the amplicon sequencing approach is approximately 100 times more sensitive than real-time PCR, with detection of amplicon specific reads even at the lowest tested spiking concentration (around 2.5–50 Colony Forming Units (CFU)/ml). Based on the results, we propose this amplicon sequencing assay as a viable alternative to replace the current real-time PCR-based singleplex assays, enabling higher-throughput biodefense applications.

HO 04 ☣ 12+3min

Wastewater testing as a tool of monitoring and controlling infections

D Frangoulidis[1], D Barth[1], R Markt[2], E Mantel[3], G Großmann[1], S Albrecht[1], K Roßmann[1]
1- Bundeswehr Medical Service Headquarters VI-2, Medical Intelligence and Information, Munich, DEU; 2- Carinthia University of Applied Sciences, Klagenfurt, AUT; 3- Bundeswehr Institute of Microbiology, Munich, DEU

Although it has been known for a long time and has been an established method for more than 20 years, especially within the framework of the WHO polio eradication programme, the examination of wastewater for infectious agents only gained momentum with the worldwide SARS-CoV-2 pandemic. As an important element in monitoring the dynamics and heterogeneity of COVID-19 infection in a population and also the success of protection and containment measures, this procedure is uniquely successful and an integral part of control and containment measures.

For two years, the German Armed Forces have established a corresponding sewage monitoring system in the MINUSMA mission in GAO (MALI) to detect SARS-CoV-2-RNA and have been able to gather extensive experience and knowledge, which is presented here. Not only was it possible to identify numerous infection clusters at an early stage and thus to initiate diagnostic, isolation and other protective measures at an early stage, but daily sampling at different locations also made it possible to trace and contain infection incidents in a timely manner, which previous methods could not achieve. Wastewater analyses for infection parameters are thus an important method of monitoring, surveillance and outbreak detection and control, which should be used as a permanent method in many medical areas in the future.

HO 05 ☣ 12+3min

Tracing pathogen evolution and epidemiology using Tree / Forest Graphs

D Lang[1], M Bestehorn-Willmann[1], H Sill[1], E Mantel[1], S Zange[1], JM Riehm[2], G Dobler[1], MH Antwerpen[1]
1- Bundeswehr Institute of Microbiology, Munich, DEU; 2- Bavarian Health and Food Safety Authority (LGL), Department of Animal Health, Oberschleißheim, DEU

The SARS-CoV-2 pandemic once more demonstrated the necessity to delineate evolutionary clades

in order to monitor, trace and control the threats posed by pathogens. During the COVID years, phylogenetic concepts and terminology, like delta, BA.2 or BA.5.1 that normally would be confined to expert discussions in molecular cladistics and epidemiology, for months dominated headlines and even permeated the awareness of the general public. At the same time, the definition and study of these viral clades was community-driven and heavily relied on the expertise of hundreds of scientists. These labor-intensive efforts involved the manual definition of clades and mutations that enabled the AI-driven high-throughput classification with tools like PANGOLIN and Nextclade.

While they represent success stories, these approaches cannot be readily transferred to other pathogens. The underlying phylogenetics require continued manual intervention and expert knowledge and strongly depend on the quality and composition of datasets. Clearly, not all pathogens are studied on such a global scale as SARS-COV-2. Thus, applying such approaches to molecular epidemiology and bioforensics in a growing number of viral and bacterial pathogens, does not scale well.

To tackle these issues, we have developed Tree and Forest Graph algorithms which enable the automated clade definition and study of evolutionary relationships as networks of pathogen isolates that can be mined and overlaid with patient metadata using graph algorithms and other AI approaches. Main concepts of the novel forest2net approach will be introduced and its application to the study of viral and bacterial pathogens demonstrated.

HO 06 ☣ 12+3min

Taxonomic assignment of *Bacillus cereus* biovar *anthracis* — There and back again

SR Klee, S Dupke, C Gummelt, HC Scholz
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In 2001, an untypical bacterium was detected which caused an anthrax-like disease in chimpanzees. First, *Bacillus anthracis* was assumed as causative agent and the bacteria were assigned accordingly. However, further analyses revealed important differences compared to classic *B. anthracis*, which was defined as a monophyletic cluster of bacteria possessing typical features lacking in the chimpanzee strains. After many discussions, we decided on the designation '*B. cereus* biovar *anthracis*' (*Bcbva*), which was not perfect, but an acceptable compromise.

In the last years, the number of genome sequences in the databases was rising and more species of the *B. cereus* group were described. Taxonomic classification based on phenotype and disease was shifted to sequence-based taxonomy. Genomospecies were defined by Average Nucleotide Identity (ANI), which today allows classification of *Bcbva* as *B. anthracis*, together with classic *B. anthracis* and some strains of *B. thuringiensis*. To overcome this confusing taxonomy, a new system was proposed in which anthrax toxin producing strains like *Bcbva* would be designated as *B. mosaicus* biovar Anthracis (or *B.* Anthracis), to reflect their clinical relevance. Nevertheless, the NCBI database contains several environmental strains designated as *B. anthracis*, and these strains lack the virulence plasmids, the *plcR* mutation and the prophage regions. The fact that obviously harmless bacteria are called *B. anthracis* raises formal and legal problems.

HO 07 ☣ 12+3min

EuroThrax: Detection, Isolation and Bioforensics of *Bacillus anthracis* and highly-pathogenic *Bacillus cereus* sensu lato in Austria and Hungary

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1- Ministry of Defence, Armament and Defence Technology Agency, Vienna, AUT; 2- Bundeswehr Institute of Microbiology, Munich, DEU; 3- Max Planck Institute for Evolutionary Anthropology, Leipzig, DEU; 4- Institute for Microbiology, University of Veterinary Medicine, Vienna, AUT

Bacillus anthracis, a zoonotic pathogen that affects humans and animals, has gained increased prominence because of its past and recent misuse as a bioterror agent. With the ongoing climate change, the organism's habitability in European soils is possibly increasing and, as a consequence, the likelihood of natural contaminations and outbreaks is increasing as well. Data on past and current genotypes of *B. anthracis* in Central Europe, respectively Austria, is very limited. Although anthrax is historically known in Austria, only three clinical isolates from Tyrol have been sequenced so far and genetic information from environmental isolates is lacking. Archive data on anthrax in Upper Austria pointed to imports of *B. anthracis*-contaminated hides in 1913. A soil analysis conducted in spring 2023 at the historical site of the respective tannery revealed the presence and persistence of *B. anthracis* spores. Using an optimized soil isolation protocol for *B. anthracis*, we were able to retrieve viable *B. anthracis* spores from this historic site. To our knowledge, this is the first *B. anthracis* of soil-borne origin isolated in Austria. These isolates, as well as historic anthrax samples from the University of Veterinary Medicine Vienna and current Hungarian *B. anthracis* isolates, were subjected to canSNP typing to decipher their genetic relationship. Fur-

thermore, analysis at a historic anthrax outbreak site in the National Park Kalkalpen uncovered a novel B. cereus strain harboring PL3, a routinely used diagnostic marker for *B. anthracis*. In summary, our results enforce the importance of archive data analysis for gaining insights into the history of *B. anthracis*.

HO 08 ☣ 12+3min

Comparison of Illumina and Oxford Nanopore Technology for Genome Analysis of *Francisella tularensis*, *Bacillus anthracis*, and *Brucella suis*

J Linde, H Brangsch, C Thomas, MC Elschner, F Melzer, H Tomaso
Friedrich-Loeffler-Institute (FLI), Institute of Bacterial Infections and Zoonoses (IBIZ), Jena, DEU

Today, high-resolution genotyping and the detection of genetic markers are performed using Whole-Genome Sequencing (WGS). This is crucial for highly pathogenic bacteria like *Francisella tularensis*, *Brucella* species, and *Bacillus anthracis*. While Illumina short-read sequencing has been established, Oxford Nanopore Technology (ONT) long-read sequencing has yet to be evaluated for those bacteria. Three independent sequencing runs were performed for each of the bacterial species utilizing Illumina, ONT flow cell version 9.4.1, and ONT flow cell 10.4. In terms of quality and detection of genetic markers, data from ONT sequencing alone, Illumina sequencing alone, and two hybrid assembly procedures were evaluated. Furthermore, the performance of the sequencing technologies was investigated in terms of commonly used standard typing approaches as well as WGS-specific high-resolution genotyping.

High-resolution genotyping of *F. tularensis* utilizing core-genome MLST (cgMLST) and core-genome Single-Nucleotide-Polymorphism (cgSNP) typing, yielded results that were comparable between data from Illumina and both ONT flow cell versions. Only data from flow cell version 10.4 produced results comparable to Illumina for both high-resolution typing methods for *B. anthracis*. However, when comparing Illumina data to data from both ONT flow cell versions for *B. suis*, high-resolution genotyping revealed significant differences. In conclusion, combining ONT and Illumina data for high-resolution genotyping may be viable for *F. tularensis* and *B. anthracis*, but not for *B. suis*. In the future, advances in nanopore technology and subsequent data processing may make high-resolution genotyping possible for all bacteria with small mutation rates.

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

I

Diagnostics and Detection

Chairs: Kilian Stoecker (DEU) and Edmund Newman (GBR)

IO 01 ☣ 12+3min

Improving existing Rapid Response Mobile Laboratories

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The COVID-19 pandemic and various Ebola epidemics clearly highlighted the importance of rapidly deployable diagnostic capacities in order to address cross border health threats. One powerful tool for this are rapidly deployable laboratories. The Bundeswehr Institute for Microbiology developed the concept for such a rapidly deployable laboratory, which since then has become the gold standard in outbreak diagnostics and has served as blueprint for many nations to establish own capacities. However, despite the success of the system, there is still a clear need for improvement to increase preparedness for the next pandemic and cross border health threats. For instance, the existing rapidly deployable laboratories only have limited sample throughout capacities. Previous experiences illustrated that such systems can process a maximum of 150 samples per day only. Furthermore, until now there is no possibility for deployed labs to quickly react to emerging pathogens or variants of concerns detected by sequencing. The overall aim is the establishment of a new rapidly deployable outbreak investigation asset. We aim to evaluate and combine existing automatization systems for the extraction of nucleic acids and master-mix preparation in order to increase the throughput of the deployed laboratory. Bringing such robotic systems to the field for the first time will presumably facilitate processing of several hundred samples per day. Furthermore, we will develop a field suitable workflow for the Kilobaser, the world's first microfluidic chip and cartridge based DNA and RNA synthesizer. Here we will show our first experiences with a few new

developments gathered during the Exercise Precise Response in Canada.

IO 02 ☣ 12+3min

How shotgun proteomics can be used as a general method to detect viruses

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1- TNO, CBRN protection, Rijswijk, NLD; 2- TNO, Microbiology and Systems Biology, Leiden, NLD; 3- HORAIZON Technology BV, Delft, NLD

Recent outbreaks of SARS-CoV-2 and Monkeypox shows the pitfall of diagnostics merely aimed at a one or at best a limited number of agents. To increase the number of virus species that can be detected we developed a shotgun proteomics based approach. Thereby, shotgun proteomics was applied on viral samples. In short, samples are inactivated and proteins in the sample were purified and digested with trypsin. Next, MS-spectra were generated on a Orbitrap instrument coupled to a nano-LC. Data was processed in PEAKS X using a combination of *de novo* sequencing and database searching. Next, the identified peptides were searched for in a database with 1895 viruses including strain variants using a web application (https://www.proteome2infection.com).

The developed shotgun proteomics method identifies a virus based on several (≥ 3) species specific peptide sequences. In 100% (42/42) a virus was detected in 40 and 2 of the cases the viruses were identified to the species and family level, respectively. Viruses identified included SARS-CoV-2, West Nile virus, Influenza A and Vaccinia virus amongst others).

Advantages of the developed method are that in a single analysis 1895 virus species including strain variants can be identified within eight hours. The assay is executed without virus specific reagents and is relatively insensitive to genetic mutations.

In conclusion, the presented proteomics based approach demonstrates that a variety of clinically relevant viral species can be identified through single shotgun proteomic analysis, indicating that mass spectrometry is a promising agnostic tool to identify viruses. Implementation of this diagnostic platform will help to prevent us from being surprised by emerging biological threats in the future.

IO 03 ☣ 12+3min

Autonomous, unattended air-monitoring system for early stage detection of biological threats by combining C-FISH and a novel air sampling technology

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Introduction: Development of a demonstrator for autonomous, unattended air-monitoring for early stage detection of biological threats. It should result in a reliable system with remote operation capabilities, autonomous monitoring and early warning functionality. *Bacillus anthracis* served as a target agent; both as vegetative cells and as endospores.

Materials and methods: Autonomous computer-controlled fluorescence in situ hybridization (C-FISH) using specific Cy3-labeled DNA probes was combined with vapor-injected semi-cyclone-air-sampling (VISCAS) technology. This system with a direct interface to a unique air-sampling (filter-free) module was developed and tested. For safety reasons *Bacillus thuringiensis* was used as model agent.

Results: The demonstrator is developed to detect *Bacillus thuringiensis* vegetative cells and endospores in air under the following performance parameters:

* Limit of Detection of 0.1 ACPLA * Time-to-Result (in-field and unattended) 45-180 minutes. * Sampling speed of 1250 liter/min * Air capacity of 5-20 m^3/ test * Sensitivity image analysis of 99,5% * *In-silico* specificity DNA probes 100% * *Expected reliability of* >99,997%

Conclusions: The demonstrator yields a promising and fully functional system for rapid, autonomous and in-field monitoring of air for *Bacillus anthracis* simulants.

Practical relevance: The system can be expanded by choosing DNA probes with different specificities in order to detect other relevant bioorganisms enabling the detection of various biological agents, ranging from *Bacillus anthracis*, *Yersinia pestis* and *Coxiella burnetii* to Smallpox and Marburgvirus. A fieldable prototype l is in actual design phase.

IO 04 ☣ 12+3min

Detection of *Vibrio cholerae* from drinking water - possibilities and challenges

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Vibrio cholerae is ubiquitous in aquatic environ-

ments, primarily in marine waters, *V. cholerae* also grows in drinking water. Cholera outbreaks occurs worldwide, often it is endemic in temperate to tropical regions. Large outbreaks with huge numbers of cases often follow natural disasters or crisis situations. Besides this, possible acts of sabotage or terrorism have to be considered not only from the military point of view, but also for attacked populations. Human infection with V. *cholerae* usually occurs after ingestion of contaminated food or drinking water, leading to massive diarrheal illness. Through its massive fecal shedding, the direct detection of *V. cholerae* from clinical samples is possible even by direct microscopy. In contrast to this, the identification of possible infectious sources, the absence in 100 ml water or 25 g food after 24 to 48 hours is a generally accepted international standard.

The fast and reliable exclusion from drinking water has a high significance under critical circumstances. The aim of this study was to validate a fast and sensitive exclusion protocol using a combined cultural-molecular diagnostic approach. After filtration of 100 ml drinking water with subsequent enrichment in 40 ml alkaline peptone water with qPCR-based detection, the current protocol enables the sensitive exclusion in less than 6 hours. The ongoing optimization should facilitate the exclusion in an even shorter time, usable under field conditions.

IO 05 ☣ 12+3min

❯ QIAstat open Cartridge: A universal tool to rapidly develop targeted panels

S Reister[1], D Lueerssen[2], J Junyent[3], A Ho[4], A Molins[3]
1- QIAGEN, Product development MDx, Hilden, DEU; 2- QIAGEN, Product development MDx, Barcelona, ESP; 3- QIAGEN, Global Systems Solutions, Barcelona, ESP; 4- QIAGEN Sciences LLC, Molecular Diagnostics, Germantown, MD, USA

Syndromic testing platforms have big advantages on the needed user training level and the ability to deliver up to 40 results for one sample within a short time. Contrary to this, these platforms are not flexible and only fixed panels are available making it difficult to react on changed target needs or sample types.

Here we present a hybrid concept the QIAstat open Cartridge. It contains all reagents needed for the Sample preparation including a full process control, as well as reagents needed for downstream one-step PCR. The Reaction chambers later on used for the PCR amplification are in contrast to a standard product only sealed on the back, allowing the collaboration partner to add their own assays, dry them at room temperature of 37°C and seal them using a heat sealer. Afterwards the Cartridge can be directly used or stored in a sealed aluminum pouch with a room temperature shelf life of several month.

The product can be used in a standard QIAStat instrument, and this concept was used as development vehicle for all panel developments QIAStat has developed in the past and is currently pursuing. The sample Prep frontend is compatible with a multitude of difficult sample types like full blood or wastewater.

In summary, this concept allows the combination of both worlds, generating integrated flexibility.

IO 06 ☣ 12+3min

❯ The mobile laboratory movement for molecular analytics

J Silvery, A Kofoet, C Tiemann
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For a flexible analysis, especially in times of a crisis with compromised infrastructure, it could be important to be able to perform analytics outside a stationary laboratory. For this purpose, we were provided with the so-called "Mobile Station"[1]. Currently, only two such trailer based mobile stations are established in Europe, one at our site and a second one in Ukraine.

In this laboratory all technical equipment and consumables necessary for PCR analysis are available. A spatial separation of pre- and post-application areas has been realized. In order to evaluate this system solution, we carried out corona diagnostics there over a period of several months. As a result, the mobile laboratory capacity could be determined at least for 1000 samples per day.

Within the framework of various cooperation projects, the laboratory was also presented to external partners and staff from other institutions were trained. For the WHO, for example, we familiarized colleagues from the Ukraine with the mobile laboratory over a period of one week. An identical laboratory is already in Ukraine and will replace destroyed stationary structures there. The laboratory is operated there as a self-sufficient facility with its own energy supply and provides infectious disease analysis for a larger regional area. In other projects, the laboratory is being used as a template for a system solution for the Armed Forces in order to be able to maintain mobile operational structures in the event of future disasters and for biological hazardous material defense. In principle, the mobile laboratory allows the availability of molecular analytics independent of location, even in a developed

infrastructure. This means that PCR testing can be carried out directly on site, for example in the context of outbreak events, and the time required for transport and sample logistics can be saved.

We present here the results of the on-site evaluation and describe the functioning and experience in using a mobile laboratory unit that we have been able to gather over the last few months.

[1]"Mobile Station" is a name of the company Seegene (Korea).

IO 07 ☣ 12+3min

Developing the UK Public Health Rapid Support Team Flight Case Laboratory as a tool for outbreak response, capacity strengthening and research

C Leggio, DP Carter
UKHSA and LSHTM, UK Public Health Rapid Support Team, London, GBR

Rapid response mobile laboratories (RRMLs) play a vital role in responding to health threats by providing rapid pathogen diagnostics and characterisation at the epicentre of an outbreak. Following the 2013-14 Ebola outbreak, the UK-Public Health Rapid Support Team (UK-PHRST) was established and, in 2018, the flight case laboratory (FCL) was added to UK-PHRST's offering. The FCL is a type II RRML which is a box-based laboratory suitable for rapid deployment by air and road transported by a team of scientists to respond to outbreaks overseas. As well as providing surge diagnostic capacity during outbreaks, the remit of the FCL has evolved to include operational deployments with a multidisciplinary approach for capacity strengthening and research in collaboration with ODA eligible partners.

Reshaping the role of RRML during peace time remains a key topic for discussion amongst all RRML owners and the UKPHRST has adopted a more flexible use of its RRML capacity and to embed this into the activities spanning across its triple remit of outbreak response, research and capacity strengthening. To achieve this, the FCL has been upgraded with portable but high specification equipment such as high-throughput ONT sequencing and syndromic panel platforms. These modules of the laboratory, as well as the existing modules such as serology instruments and isolators, can be deployed for use in across a variety of project as well as being available for use in outbreak response. This enables rapid operational research projects to start as soon as funding is approved as procurement of complex equipment can often take several months and, with the use of high specification equipment, would also allow capacity strengthening and research projects to be more ambitious and provide more comprehensive data than before. The UKPHRST flexible team structure also enables it to design and deploy specialised training covering high containment, sequencing, and molecular diagnostics to meet partners' requests.

IO 08 ☣ 12+3min

Development of Minimum Operational Standards for Rapid Response Mobile Laboratories (RRML)

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World Health Organization WHO, Regional Office for Europe, Copenhagen, DNK

Rapid Response Mobile Laboratories (RRMLs) provide critical laboratory diagnostic support in crisis situations and are an important asset of the global emergency workforce. In 2018, the Health Emergencies Programme at the WHO Regional Office for Europe (WHE/EURO) established the RRML Initative, driven by European Global Outbreak and Alert Network (GOARN) partners, to integrate RRMLs into existing preparedness and response structures and to standardize RRML operations in the field.

To strengthen RRML capacities and coordination, a conceptual framework was developed to support RRML activities and establish minimum operational standards (MOS). These standards function to provide a rapid, targeted and quality operational response in the field, supporting affected communities on national, regional, and global level. Experts from all over the world defined RRML MOS to be applied across all phases of the RRML deployment lifecycle, from the initial request for assistance to the end of mission, and addressing RRML main areas of work and coordination processes. In addition, a RRML Monitoring and Evaluation (M&E) System was developed based on MOS as a pilot for a forthcoming WHO recognition process for RRMLs.

Recently an Interregional Field Simulation Exercise (IFX) was held in Istanbul, Türkiye as part of a simulation exercise programme for RRML. The event brought together RRMLs and Emergency Medical Teams (EMTs) from 32 countries from all WHO Regions to validated comprehensiveness, applicability, and feasibility of proposed MOS applying the RRML M&E System and to explore in-field coordination procedures.

A Interregional Meeting held in conjunction with the IFX also set the basis for a global RRML network, with the engagement of partners and stakeholders across all WHO Regions and reflective of a broad range of existing networks and initiatives, to support expansion of this technical community of practice.

The outcomes of the IFX will support the publication of the RRML MOS as a global standard setting product and enforces the engagement of global partners in the RRML network as a driving force for RRML development and emergency response.

Audimax / 11:00 ... 12:30

J Medical Countermeasures - Immunology and Vaccines

Chairs: Heiner von Buttlar (DEU) and Xavier De Bolle (BEL)

JO 01 ☣ 23+7min

Structure and growth of the envelope in the *Brucella abortus* pathogen

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Bacteria of the *Brucella* genus are collectively responsible for a worldwide zoonosis called brucellosis. *Brucella abortus* is a Rhizobiale, part of the alphaproteobacteria. We found that the most abundant outer membrane proteins, Omp2b and Omp25, are attached to the peptidoglycan by a N-terminal extension, with a short conserved motif that is critical for covalent linkage to *meso*-diaminopimelate. In the absence of such linkages, the outer membrane blebs. We identify a key enzyme required for these linkages, a L,D-transpeptidase called Ldt4.

The outermost structure of the envelope is the lipopolysaccharide (LPS). In *B. abortus* LPS is known to be poorly immunogenic and to be characterized by a branched structure in the core. The presence of an O-chain is crucial to avoid brutal phagocytosis by macrophages, and we identified the O-chain ligase as WadA, an original enzyme with two active sites on opposite sides of the inner membrane. Indeed, WadA can modify the core in the cytoplasm (as previously shown) and add the O-chain in the periplasm. Our data show that LPS is inserted at the new pole and then at the division site during the cell cycle. Since LPS is translocated from the inner membrane to the outer membrane by a Lpt (LPS transport) system, we have localized components of this complex and we found that insertion sites of LPS correlates with the position of Lpt proteins anchored in the inner membrane.

Localized insertion of LPS can be demonstrated by the addition of 2-deoxy-D-manno-octulosonic acid (KDO) conjugated at an azido group at position 8 (KDO-N_3). We also show that mannose-N_3 (with an azido group at position 6) can be incorporated at a specific position of the LPS, in the lateral branch of the core. We have also identified the enzymatic pathway that allows the incorporation of mannose-N_3.

A better understanding of the fundamental processes that allow the assembly of the *B. abortus* envelope will lead to new opportunities to combat this nasty but successful pathogen.

JO 02 ☣ 12+3min

Differentiation between Mpox infection and Imvanex immunization by a novel machine learning supported serological multiplex assay

R Surtees[1], M Grossegesse[1], F Treindl[2], M Skiba[2], T Rinner[2], BG Dorner[2], A Nitsche[1], D Stern[2]
1- Robert Koch Institute, Highly Pathogenic Viruses (ZBS 1), Berlin, DEU; 2- Robert Koch Institute, Biological Toxins (ZBS 3), Berlin, DEU

Serological assays are crucial for epidemiological studies on burden of disease, underreported infections or outbreak analysis in populations at risk of infections. However, if serological assays are used to detect infections for a disease where vaccines are available, differentiation between the humoral immune response induced by infection from vaccination is essential but not always easily implemented. Here we describe a novel serological multiplex assay which is able to discern the humoral immune response after Mpox infection from the immune response after Imvanex immunization. To this aim, 14 recombinant proteins derived from Mpox (MPXV) and Vaccinia virus homologue membrane proteins as well as the A-type inclusion protein from Cowpox virus (CPXV), and whole cell lysate from Vaccinia virus VR-1536 infected cells were combined in a bead-based multiplex suspension array based on the Luminex platform. The assay was highly sensitive and correlated well with established serological assays such as immunofluorescence and neutralization assays. Three different linear discriminatory analysis models were trained on IgG multiplex results on four different patient panels: childhood-immunized or naïve, Imvanex immunized, CPXV infected, or MPXV infected. The assay was highly efficient

in differentiating between immunized and infected sera with balanced accuracies of up to 99.5% for sera after Imvanex immunization and up to 99.1% after Mpox infection, indicating that our assay is a powerful tool for both epidemiological studies and Mpox diagnostics.

JO 03 ☣ 12+3min

Broadly neutralizing polyclonal antibodies to combat biological agents in emergency situations

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Fabentech's company has over 14 years of expertise in the development and production of medical countermeasures. Its technology relies on the production of broadly neutralizing antibodies that neutralize targeted viruses or toxins, and their variants to halt disease progression.

We propose an overview of our programs, showcasing *in vitro* and *in vivo* studies of our treatments against acute viral diseases and intoxications.

Fabenflu®, a fully developed post-exposure treatment of H5N1 avian influenza infection in humans. Treatment showed its ability to neutralize over 20 viral strains of the highly pathogenic avian flu virus H5N1.

An emergency therapeutic solution targeting ricin poisoning is in development with the support of the French defense procurement agency (DGA). Our anti-ricin treatment exhibits strong neutralizing activity against toxins extracted from 7 different cultivars. Preliminary studies in intoxicated mice and non-human primates demonstrated its efficacy.

Treatment of hospitalized immunocompromised COVID-19 patients with an ongoing phase 2 study. This treatment showed cross-neutralization against all tested variants of Sars-Cov-2 up to Omicron BQ.1.1 and XBB.1.5.

Programs to develop antidotes against Abrin poisoning, a plant-based toxin, and Nipah virus infection.

Immunotherapies based on polyclonal antibodies offer a reliable solution to neutralize the most critical biological agents, providing a crucial defense against pandemics or potential bioterror attacks.

JO 04 ☣ 12+3min

Development of a Nipah measles vector vaccine (MV-NIV) to be used in outbreaks situation

B Pályi[1], Z Kis[1], D Deri[1], J Henczkó[1], N Magyar[1], P Hajdrik[1], DG Horvath[2], GK Balka[2], A Richard[3], T Fujiyuki[4], M Yoneda[4], C Kai[4]
1- National Public Health Center, National Biosafety Laboratory, ERINHA node, Budapest, HUN; 2- University of Veterinary Medicine, Department of Pathology, Budapest, HUN; 3- European Research Infrastructure on Highly Pathogenic Agents (ERINHA-AISBL), Brussels, BEL; 4- University of Tokyo, Institute of Industrial Science, Tokyo, JPN

Introduction: Nipah virus causes infections that exhibit a variety of signs, from asymptomatic cases to case fatality rate up to 40-90%. The WHO Blueprint has identified an urgent need for developing countermeasures against Nipah virus (NiV), including vaccines that are protectives and have a rapid onset of immunity.

Materials and methods: The MV-NiV vaccine candidate, developed by the University of Tokyo, is based on the Measles virus Edmonton strain with NiV (strain Malaysia) glycoprotein as the antigen, which is considered as the most immunogenic antigen and also mediates the virus receptor binding. In this proof of concept study, beside the placebo group, hamsters were immunized using two different doses of MV-NiV vaccine and challenged with NiV Malaysia strain under BSL-4 conditions. The protection was characterized by survival rate, virological measurements, antibody titers and immunohistological (IHC) staining.

Results: Immunization using a higher dose of vaccine resulted in a 100% survival rate, with superior neutralization antibody titers and no sign of IHC positivity. Both vaccine doses protected the animals from significant weight loss, with the higher dose also preventing severe clinical signs. The neutralization antibodies showed cross protection to NiV (Bangladesh strain). Based on these results, the MV-NiV vaccine protect the animals form Nipah virus infection.

This work has been funded by CEPI.

JO 05 ☣ 12+3min

Preclinical models for the assessment of vaccine efficacy against hazard group 4 pathogens

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UKHSA, Porton, Salisbury, GBR

Emerging viral pathogens continue to cause major public health concerns to human populations. To rapidly be able to respond to these threats, we need to understand the disease pathogenesis, the natural progression of infection and host response. For this purpose, *in vivo* models have been developed, which

can answer the above concerns but in addition have value in assessment of intervention strategies including vaccine development.

We have established animal models for several viral haemorrhagic fevers caused by hazard group 4 pathogens including Crimean-Congo Haemorrhagic Fever, Nipah, Lassa, Marburg and Ebola viruses which are classified as CDC/NIAID Bioterrorism agents. For some models the virus has required host adaptation to increase disease severity whereas for others transgenic mice have been utilised. Due to the requirement of containment level 4 laboratories for the handling of these live pathogens, the facilities at the UK Health Security Agency are one of only a few available capable for assessing vaccine efficacy with live challenge virus.

These models have proven their value in testing the efficacy of vaccine candidates being developed, including differentiating the best constructs and supporting the progression of promising approaches into human clinical trials. Given the epidemic potential for many of these pathogens, and their inclusion as priority pathogens by many strategic groups (e.g. WHO, CEPI, UK Vaccine Network), these models are available for testing and will be refined according to the pathogen landscape.

Garden Hall / 11:00 ... 11:45

K

Open Topics

Chairs: Gregor Grass (DEU) and Mats Forsman (SWE)

KO 02 ☣ 12+3min

Preparing for biological weapons investigations: Capstone Exercise 2020/2022 and its implications for future training of qualified experts on the UNSGM roster

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In times of ongoing severe conflicts in the world, attacks with biological weapons are a threat that are monitored with concern which is why several states have re-emphasized the need to strengthen the United Nations Secretary-General's Mechanism for Investigation of Alleged Use of Chemical and Biological Weapons (UNSGM).

Germany is one of the states supporting the UNSGM with trainings for qualified experts that are listed for potential investigation missions. In this context, the Robert Koch Institute organized the Capstone Exercise 2020/2022 with funding from the Federal Foreign Office. With the aim to identify training potentials and to observe the interaction of different UNSGM stakeholders, 19 qualified experts had the opportunity to take part in this full-scale simulation exercise where they planned and executed a mission based on a fictitious scenario. An evaluation team of international observers made various recommendations regarding *inter alia* the preparedness of the mechanism, coordination between the different stakeholders (including the UN and analytical laboratories), and necessary capability-building for qualified experts. Future training should be based on a structured approach focusing on the various skills needed for an investigation, such as sampling, interviewing, command and control, and security awareness. Under the coordination of the UN Office for Disarmament Affairs, the lessons learned of the exercise will be used to strengthen the UNSGM.

KO 03 ☣ 12+3min

Peroxyacetic acid (PAA) based aerosol decontamination of commercial aircrafts

J Kohs[1], S Klafack[1], KA Schwenke[2], P Siller[3], J Reißner[4], H Freese[1], A Below[1], T Lübcke[5], S Mehler[6], B Albert[6], S Hölterhoff[6], H Barth[6], M Streitz[1], M Engel[7], J Schinköthe[8], M Thanheiser[9], U Rösler[4], S Reiche[1]
1- Friedrich-Loeffler-Institute (FLI), Department of Experimental Animal Facilities and Biorisk Management (ATB), Greifswald - Insel Riems, DEU; 2- Robert Koch Institute, Biosafety Level-4 Laboratory (ZBS 5), Berlin, DEU; 3- Federal Office of Consumer Protection and Food Safety, Berlin, DEU; 4- Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, DEU; 5- Lufthansa Technik, Hamburg, DEU; 6- Lufthansa Technik, Frankfurt, DEU; 7- AKUT SOS CLEAN GmbH (SOS), Frankfurt, DEU; 8- Leipzig University, Institute of Veterinary Pathology, Leipzig, DEU; 9- Robert Koch Institute, Hospital Hygiene, Infection Prevention and Control (FG14), Berlin, DEU

Commercial aircrafts could play a key role in the emergence of pandemics, but validated, efficient room decontamination procedures that maintain

the airworthiness of an aircraft are still lacking. The particular challenge is to identify effective disinfectants and procedures that are compatible with all installed materials and electronics.

For this purpose, we nebulized peroxyacetic acid (PAA) into ultrafine aerosol particles of about 7.5 μm to generate a so-called 'dry fog'. A wide range of different microorganisms were treated with PAA aerosols for 3 h or 2x 90 min with additional interim ventilation. After determination of the minimal effective PAA concentration and intensive investigations of material compatibility by Lufthansa Technik (LHT), the method was tested on-site in an Airbus A320 of the LHT.

Due to the application as aerosol, the corrosive properties of PAA can be largely avoided. Depending on the PAA concentration, exposure time, relative humidity and temperature, a microorganism reduction of at least 4 log10 steps can be achieved. Thereby, the 'dry fog' method resembles the first validated, semiautomatic room disinfection procedure, which can be combined with the established manual wet-chemical surface disinfection procedures in aircrafts after contamination with highly pathogenic agents of human or veterinary relevance.

KO 04 ☣ 12+3min

❯ *Francisella tularensis* survival in natural water implicate revival in susceptible hosts for persistence

J Thelaus[1], D Birdsell[2], S Bäckman[1], M Granberg[1], E Salomonsson[1], I Golovliov[3], E Lundmark[1], D Sundell[1], J Näslund[1], L Noppa[1], AL Johansson[1], K Wallgren[1], O Rzhepishevska[4], JD Busch[2], JW Sahl[2], A Johansson[3], M Forsman[1], DM Wagner[2]
1- Swedish Defence Research Agency, Division of CBRN Defence and Security, Umeå, SWE; 2- Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA; 3- Umeå University, Department of Clinical Microbiology and the Laboratory for Molecular Infection Medicine Sweden, Umeå, SWE; 4- Umeå University, Department of Chemistry, Umeå, SWE

An epidemiological connection of *Francisella* to aquatic sources is well documented. We hypothesized that the natural life cycle of tularemia includes a persistent host independent state in water and infection in mammals is a mechanism for *F. tularensis* to switch back and forth between vegetative and persistent states. To this end, we studied the persistence of virulent *F. tularensis* Type A and Type B in water. After one year in nutrient poor, axenic and cold conditions (4°C), strain FSC200 (Type B) was still culturable and fully virulent in mice. Strain SchuS4 (Type A), even though non-culturable after one year, was still causing tularemia in mice. During transition to low-nutrient conditions in cold water, FSC200 displayed a different metabolomic profile, as compared to SchuS4. In an experimental set-up, using natural pond water neither FSC200 nor Schu S4 persisted in environments with active competitive bacterial growth at room temperature, but could maintain virulence for at least 10 weeks in colder temperatures.

We show that aquatic free-living and non-replicative *F. tularensis* is under a steady decline. Still, remaining cells are able to cause disease in mice. Both water temperature and microbial competition influence for how long *F. tularensis* populations are able to persist and avoid extinction in between outbreaks. The results brings new perspectives on the low infection dose, the wide host-range and the wide range of transmission routes exploited by this bacterium.

Audimax / 13:30 ... 15:30

L

Strategies, Concepts and Analyses

Chairs: Roman Wölfel (DEU) and Lisa Hensley (USA)

LO 01 ☣ 25+5min

❯ German-Georgian Collaboration for Georgian Public Health System Strengthening

A Gamkrelidze
Former Director General of National Center for Disease Control and Public Health, Tbilisi, GEO

Cooperation between the Bundeswehr Institute of Microbiology and the National Center for Disease Control and Public Health (NCDC) spans ten years. Partnership was developed with supported of German Agency for International Cooperation, in the frame of Biosecurity Program, bringing significant results for Georgian scientific public health practice. Project aim was to strength national capabilities to rapidly identify, assess dangerous pathogens and respond to biological threats. Capacity enhancement

included implementation of state-of-the-art methods to improve diagnostic capabilities, especially important during the COVID-19 pandemic.

German-Georgian relations traced back more than two hundred years. Historically, collaboration and contribution of Germany became vitally important, especially for institutional, economic, cultural and scientific transformation. Notably, German cooperation in medicine and pharmacy begins from 18^{th} century and is longest standing. After declaring independence, Germany was first country establishing diplomatic relations and one of the principal bilateral donors.

Germany contributed to the development Georgian public health, health insurance, bioinformatics, medical education and technologies, pharmacy, allergology-immunology, tuberculosis, cardiac surgery, urology, dialysis, audiology, children's oncohematology, anthroposophical and integrated medicine. Cooperation and achievements contribute to further strengthening of public health in Georgia.

LO 02 ☣ 12+3min
❯ Out-pacing the next chem-bio threat

SJ Calderwood
Defense Threat Reduction Agency, US Dept of Defense, Fort Belvoir, VA, USA

In just a few months, SARS CoV-2 illuminated the challenges with recognizing an emerging disease, diagnosing the condition, and managing its spread. In response, the US Government shifted its diagnostics landscape to better prepare for such a pandemic in the future.

Groundbreaking strides in science often stem from one simple question: 'what if?' Throughout the pandemic, our team asked: 'what if we could better diagnose emerging diseases?' 'What if we could produce tests faster?' 'What if we could test for pre-symptomatic disease?' As a result, the Medical Diagnostics Division of the Defense Threat Reduction Agency's (DTRA) Chemical and Biological Technologies Department is investing in solutions addressing these very questions. This division develops innovative diagnostic capabilities for the US Military via direct funding from the Chemical and Biological Defense Program to the Joint Science & Technology Office, located within DTRA. Through increased investment in minimally and non-invasive sampling methods, our division has become a lead in the agnostic diagnostics space. Currently funded efforts in this area include: continuous monitoring devices able to catch deviations in health standards more quickly; synthetic binding molecules able to rapidly and economically produce more shelf-stable assays for emerging diseases; pre-symptomatic saliva-based assay development; and soon, a novel assay able to determine whether an infected person is contagious to others, or not.

LO 03 ☣ 12+3min
❯ Anthrax bio-threat: A question of balance

DM Sabra, A Krin, AB Romeral, JL Frieß, G Jeremias
Hamburg University, Centre for Science and Peace Research (ZNF), Hamburg, DEU

Bacillus anthracis is one of the most relevant bioweapon agents. Our theory-based study with a newly developed methodology investigates how emerging and enabling technologies could both advance and undermine the biosecurity threat by *B. anthracis*. On the one hand, intertwined fields of advancing science and technology could ease the accessibility, development, and deployment of an anthrax bioweapon agent. Considering state-of-the-art and emerging technologies, we analyze all necessary steps from acquiring the bioagent to its dissemination. These include plasmid construction via DNA synthesis, solid-state fermentation for mass production, identifying aerosolization methodologies, and UAV-delivered deployment. On the other hand, the interplay of the same converging technologies is key to protecting against, for example, a bioterrorist anthrax attack, increasing the ability to react during such an event, and to mitigate its subsequent impact. Important novel countermeasure capabilities strengthening biodefense proficiencies are based on advances in diagnostics, improved treatment, innovative methods for decontamination, and state-of-the-art forensics. Analyzing the impact of enabling and converging science and technology allows for the development of effective and sustainable preparedness and necessary biosecurity architecture adaptation.

LO 04 ☣ 12+3min
❯ [pending]

R Von Tersch
USAMRIID, Chief Science Officer, Fort Detrick, Frederick, MD, USA

[pending]

LO 05 ☣ 12+3min
❯ COVID-19 Lessons Learned for NATO Bio-responsiveness Capability

J Burr, A Farris, C Scheible
Institute for Defense Analyses, Alexandria, VA, USA

Guided by the lessons learned function of the NATO CBRN Medical Training Panel, the US Institute for Defense Analyses (IDA) undertook a project to promote development of a program of work to learn lessons from the COVID-19 pandemic to improve NATO's bio-responsiveness capability. To do so, the IDA team adapted the NATO Medical Lessons Learned process to assess observed COVID-19 pandemic response challenges and best practices within the framework of the NATO Smart Defence Project (SD 1.1045) on Bio-responsiveness. We collected observations from official NATO lessons learned repositories and documented COVID-19 response collaboration teleconferences, and assessed them using metadata tagging and a combination of directed and conventional content analysis. We then collated the outputs of the analysis into a prioritized set of Lessons Identified.

The results of this initiative can be used to generate a roadmap and recommend responsible bodies to improve NATO bio-responsiveness capabilities in the Doctrine, Organization, Training, Materiel, Leadership, Personnel, Facilities, and Interoperability domains at the tactical, operational, and strategic levels. Additionally, the methodology developed to conduct this analysis can be adapted for use in other lessons learned processes and projects.

LO 06 ☣ 12+3min

Forces Readiness: Evidence to Decision about necessary vaccinations — Applying GRADE and WHO-INTEGRATE as a systematic approach to evaluate FHP decision making

K Roßmann[1], B Strahwald[2], K Kehe[3], S Albrecht[1], P Gilliot[1], T Morwinsky[1], A Ziegler[1], F Rieck[1], D Frangoulidis[1], K Erkens[1]
1- Bundeswehr Medical Service Headquarters VI-2, MI2, Munich, DEU; 2- Pettenkofer School of Public Health, Munich, DEU; 3- Bundeswehr Medical Service Headquarters VI, Koblenz, DEU

Vaccination is a critical public health intervention that has revolutionized disease prevention and control worldwide – also for military forces and their readiness and resilience. The decision to use vaccines involves complex considerations, necessitating robust evaluation frameworks to inform policy and practice. This abstract explores the power and benefits of employing the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) criteria and the WHO-INTEGRATE (INTEGRATe Evidence) system for grading decisions related to vaccination in the German Armed Forces. The GRADE criteria – e.g. in Germany applied by the STIKO of RKI for the national immunization recommendations - provide a comprehensive approach to evaluating the quality of evidence, considering factors such as study design, risk of bias, precision, consistency, and directness. By applying the GRADE criteria, decision-makers can assess the certainty of evidence supporting vaccine interventions. This systematic process helps inform the strength of recommendations and facilitates transparent communication of the evidence base, aiding policymakers and healthcare professionals in making informed choices. These recommendations developed by STIKO/RKI are used as a base for a consideration process by MI2 – supported by the Pettenkofer School of Public Health LMU - in order to choose compulsory vaccinations for the Bundeswehr forces. In parallel, the WHO-INTEGRATE system offers a dynamic framework for decision-making in public health. It combines evidence-informed decision-making with contextual factors, stakeholder engagement, and innovative approaches to generate policy recommendations. The WHO-INTEGRATE system promotes a multidimensional assessment of vaccines, considering aspects such as disease burden, cost-effectiveness, health system capacity, equity, and feasibility. By incorporating these diverse factors, the system enables tailored and context-specific vaccination strategies. This approach is now applied for COVID-19-Vaccinations in the German Armed Forces for the first time. The combination of the GRADE criteria and the WHO-INTEGRATE framework offers a robust approach to grading vaccination decisions also in military forces. Their synergy provides decision-makers with a comprehensive framework for evidence assessment and policy formulation.

LO 07 ☣ 12+3min

CBRN medical counter-measures intelligent stockpiling

B Queyriaux[1], S Kowitz[2], D Lacassagne[2], A Lambert de Rouvroit[3]
1- HIPS Agency, Munich, DEU; 2- MMCC/EMC, Koblenz, DEU; 3- Health Security Associates, Paris, FRA

CBRN medical countermeasures stockpiling is the storage of medical supplies needed to respond to a natural, accidental or intentional CBRN event.

Its goal is the availability of critical resources to protect public health and mitigate the strategic impact of a CBRN event.

Several techniques of stockpiling can address this acute unbalance between demand and offer, from classical storage of supplies combined with effective logistic to timely deliver the right quantities at the right place, to virtual or even intelligent stockpiling.

Virtual stockpiling is a network of connected and

coordinated stockpiles and logistics, surpassing the capabilities of individual isolated stockpiles when responding to CBRN events. It is today the EU approach for the protection of populations, steered by the Health Emergency Response Authority (HERA).

Intelligent stockpiling takes the concept further by incorporating industrial, legal, intelligence and logistics assets enabling a surge in pharmaceutical production, procurement and delivery in coordination with wide virtual stockpiling according to present and anticipated CBRN risks and threats. Adapted to current security challenges, it enhances military preparedness to CBRN event at home or in deployments worldwide.

Within Europe, this intelligent stockpiling would address military requirements and complete EU's preparedness to CBRN events. It involves military and civilian, security and health, public and private actors. This complex partnership needs a robust continuous command and control to ensure preparedness, readiness and response implementation. Depending on MMCC/EMC participating nations' agreement, this could be a new mission for the Multinational Medical Coordination Centre / European Medical Command in Koblenz.

Audimax / 16:00 ... 18:00

M

Case Reports (TED-Session)

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

MO 01 ☣ 20+5min

The 2022 Mpox outbreak — Experience from an institute of tropical medicine and STI clinic

M Van Esbroeck, N Berens-Riha
Institute of Tropical Medicine, Department of Clinical Sciences, Antwerp, BEL

Annually 40252 patients visit the travel and HIV/STI clinics of the Institute of Tropical Medicine (ITM) in Antwerp. More than half of the 790 Belgian mpox cases were diagnosed at ITM.

By retrospective examination of stored samples from male sexual health clinic attendees, three asymptomatic mpox cases were detected, two with replication competent virus.

Prospective low threshold screening revealed several mpox cases presenting with prodromal symptoms, lymphadenopathy or proctitis, not fulfilling the WHO and ECDC case definitions of a suspected mpox case.

By following up high risk contacts of mpox cases to better understand the transmission dynamics, the existence of transmissible mpox viral shedding in saliva and anorectal mucosal samples was detected as early as four days before symptom onset.

Mpox disease with localized severe symptoms of proctitis and penile oedema was demonstrated occurring 4-35 days after post- or pre-exposure preventive vaccination with MVA-BN vaccine.

Interim results of a prospective cohort study of mpox patients revealed that two-thirds of participants had residual morbidity, including anorectal and genital symptoms, loss of physical fitness, fatigue and mental health problems.

The rapid selection of a tecovirimat-resistant MPXV variant during tecovirimat monotherapy treatment was demonstrated in a severely immunocompromised patient with prolonged MPXV infection.

MO 02 ☣ 12+3min

Seroprevalence of *Brucella* infection in wild boars (*Sus scrofa*) of Bavaria, Germany, and associated genome analysis of *B. suis* biovar 2

C Klose[1], M Müller[2], M Hanczaruk[3], JM Riehm[3]
1- Bavarian Health and Food Safety Authority (LGL), Bacteriology, Erlangen, DEU; 2- Bavarian Health and Food Safety Authority (LGL), Pathology, Erlangen, DEU; 3- Bavarian Health and Food Safety Authority (LGL), Bacteriology, Oberschleißheim, DEU

Brucella species are highly pathogenic zoonotic agents and are found in vertebrates all over the world. To date, Germany is officially declared free from brucellosis and continuous surveillance is currently limited to farm ruminants. However, porcine brucellosis, mostly caused by *B. suis* biovar 2, is still found in wild boars and hares. In the present study, a three-year monitoring program was conducted focusing on the wild boar population in the state of Bavaria. Serologic screening of 11.956 animals and a direct pathogen detection approach, including a subset of 681 tissue samples, was carried out. The serologic incidence was 17.9%, which is in approxi-

mate accordance with previously published results from various European countries. Applying comparative whole genome analysis, five isolated *B. suis* biovar 2 strains from Bavaria could be assigned to three known European genetic lineages. One isolate was closely related to another strain recovered in Germany in 2006. Concluding, porcine brucellosis is endemic in Bavaria and the wild boar population represents a reservoir for genetically distinct *B. suis* biovar 2 strains. However, the transmission risk of swine brucellosis to humans and farm animals is still regarded as minor due to low zoonotic potential, awareness, and biosafety measures.

MO 03 ☣ 15+5min
Clinical case from North Rhine-Westphalia

D Jacob
Robert Koch Institute, Highly Pathogenic Microorganisms (ZBS 2), Berlin, DEU

Note: Due to interactive voting, no abstract is shown here.

MO 04 ☣ 15+5min
Where's the meat? A strange lymph node in a butcher

A Berger[1], A Dangel[2], T Rupp[3], HJ Mappes[3], C Schneider[4], A Sing[1]
1- Bavarian Health and Food Safety Authority (LGL), National Consiliary Laboratory for Diphtheria, Oberschleißheim, DEU; 2- Bavarian Health and Food Safety Authority (LGL), Public Health Microbiology, NGS Core Unit, Oberschleißheim, DEU; 3- Klinikum Aschaffenburg-Alzenau, Chirurgische Klinik, Aschaffenburg, DEU; 4- Klinikum Aschaffenburg-Alzenau, Zentrallabor, Aschaffenburg, DEU

Note: Due to interactive voting, no abstract is shown here.

MO 05 ☣ 15+5min
Images say more than 1000 words?

S Schneitler
Institute of Medical Microbiology and Hygiene at the University Clinic of Saarland, Solingen, DEU

Note: Due to interactive voting, no abstract is shown here.

MO 06 ☣ 15+5min
A swollen lip, itching and malaise in a traveller returning from Benin

R Spindler[1], S Zange[2], C Rothe[1]
1- LMU University Hospital, LMU Munich, Division of Infectious Diseases and Tropical Medicine, Munich, DEU; 2- Bundeswehr Institute of Microbiology, Munich, DEU

Note: Due to interactive voting, no abstract is shown here.

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

N German Biosecurity Programme

Chairs: Gordon Wilke (DEU) and Silke Bellmann (DEU)

NO 01 ☣ 20+10min
The German Biosecurity Programme

S Bellmann
Federal Foreign Office, Deputy Head of Division for Chemical and Biological Weapons Disarmament, G7 Global Partnership, German Biosecurity Programme, Berlin, DEU

1st July 2023 marked the 10th anniversary of the German Biosecurity Programme. Launched by the Federal Foreign Office as part of joint efforts of members of the Global Partnership against the Spread of Weapons and Materials of Mass Destruction to strengthen global biological security, the programme, so far, has carried out projects in more than 20 countries. It has contributed significantly to the Global Partnership's Biosecurity Deliverables: To secure and account for dangerous pathogens and toxins that represent biological proliferation risks, to develop and maintain appropriate and effective measures to prevent, prepare for, and respond to the deliberate misuse of biological agents, to strengthen national and global networks to rapidly identify, confirm and respond to biological attacks, to reinforce and strengthen biological non-proliferation principles, practices and instruments, and to reduce proliferation risks through the advancement and promotion of safe and responsible conduct in life sciences and biotechnology. Furthermore, the German Biosecurity Programme fosters cooperation and assistance among States Parties of the Biolog-

ical Weapons Convention. Currently, projects in 14 countries are implemented by the Bundeswehr Institute of Microbiology, the Robert Koch Institute, the Friedrich-Loeffler-Institute, the Bernhard-Nocht-Institute for Tropical Medicine, and the GIZ. In addition, two cross-regional projects focus on training and capacity building of scientists and researchers on a wide range of biological security topics.

NO 02 ☣ 12+3min

Mitigation of Risks of Zoonotic Diseases that May Arise as a Result of Climatic Changes in Central Asia Region

L Bakanidze, B Gulyamov
EU CBRN CoE RS for CA, Tashkent, UZB

Climate change may affect the incidence of zoonotic diseases through its effect on four principal characteristics of host and vector populations that relate to pathogen transmission to humans: geographic distribution, population density, prevalence of infection by zoonotic pathogens, and the pathogen load in individual hosts and vectors. These mechanisms may interact with each other and with other factors such as anthropogenic disturbance to produce varying effects on pathogen transmission within host and vector populations and to humans. Climate change effects on most zoonotic diseases act through wildlife hosts and vectors, therefore understanding these effects will require multidisciplinary teams to conduct and interpret ecosystem-based studies of zoonotic diseases pathogens.

Aridification of the Central Asian landscapes, the arid conditions of Central Asia determined the formation of adaptive species-specific protective behavior in vectors of zoonotic diseases. Besides, territories of the whole region are 'rich' with natural foci of causative agents of zoonotic diseases, and many of zoonotic diseases, both bacterial and viral (like plague, anthrax, brucella, CCHF, etc.) are endemic for most of partner countries. While the goal of eradicating such diseases is enticing, historical experience validates abandoning eradication in favor of ecologically based control strategies reducing morbidity and mortality to a locally accepted risk level.

Present project concept, related to mitigating risks of zoonotic diseases that may arise as a result of climatic changes in Central Asia region, has been recognized as a priority by all of the CA partner countries. The project gives opportunities for collaboration with other international and donor organizations, working in the region of central Asia, particularly, with German Biosecurity Program.

Project will be focused on improvement of understanding impact of climate change on trends of spread of zoonotic diseases, namely identification of prevalence of zoonotic diseases and their impact on human and animal health and trends of their occurrence during last years in relation with climate change, conducting studies on transboundary occurrence of outbreaks of zoonotic diseases, assessing existing available response systems, organizing joint training sessions and field works, etc.

NO 03 ☣ 12+3min

Biological Threats in Tunisia - Joint Efforts of both the Mobile Laboratory and Task Force and Decontamination Unit are essential for an effective defense

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1- Military Hospital Tunis, Department of Virology, Tunis, TUN; 2- Direction Générale de la Santé Militaire, Tunis, TUN

Tunisia is an important strategic NATO in North Africa. Increasing levels of biological threats from neighboring countries as well as increasing social instability within Tunisia make it fundamental to have a unit which is capable to efficiently disseminate these unclear biological situations from inside and outside of the country. Therefore, Mobile Laboratory and Taskforce and Decontamination Unit have been established and trained since 2017 in the framework of the German-Tunisian cooperation of the Enable and Enhance Initiative.

In order to enforce and consolidate an effective defense against unclear biological situations, the Mobile Laboratory and Taskforce and Decontamination units had a joint exercise in October 2022 to enhance the combined ability and capacity of all Tunisian Biosecurity units.

In the course of this exercise two crime scenes were investigated, an apartment of a person with symptoms of toxin poisoning and a clandestine lab. The Task Force unit took various samples, including powder, liquids, and forensic evidence, which the Decontamination unit treated for the hand-over of the samples to the Mobile Laboratory. The Mobile Laboratory analyzed the samples using protein test, gram staining, lateral flow assays and realtime-PCR to identify the pathogen.

Even in harsh and demanding environments, all pathogens were identified with 100% security in less than 5 hours. Communication and coordination between the tree operative units are essential to ensure effective analysis of the samples from crime scene to result.

NO 04 ☣ 12+3min

Seroprevalence of MERS-CoV and other zoonotic pathogens in dromedary camels in South and Southeast Tunisia

S Bauer[1], M Ben Moussa[2], A Gritli[3], H Ben Yahia[4], K Müller[1]
1- Bundeswehr Institute of Microbiology, Munich, DEU; 2- Military Hospital Tunis, Department of Virology, Tunis, TUN; 3- Direction Générale de la Santé Militaire, Hygiène et Industrie des Denrées Alimentaires d'Origine Animale, Tunis, TUN; 4- Direction Générale de la Santé Militaire, Tunis, TUN

Previous studies showed that dromedary camels are reservoirs for various zoonotic pathogens, among others Middle-East Respiratory Syndrome Coronavirus (MERS-CoV), Rift-Valley Fever Virus (RVFV), West-Nile Fever Virus (WNFV) and *Coxiella burnetii*. Dromedary camels are also an important livestock species in the Saharan regions of Tunisia and neighbouring countries. Semi-nomadic herding and movement of animals across country borders make it necessary to periodically monitor the prevalence of zoonotic pathogens in dromedary camels that could negatively affect human health and economic stability in desert regions of North Africa.

In January 2022, during mating and birthing season, we collected serum samples of more than 880 mostly female dromedary camels in the Kebili and Tataouine gouvernorates. The male dromedary camels in this study were used mainly for transport and patrol and were kept enclosed. We performed ELISA assays for antibodies against seven potentially zoonotic pathogens, MERS-CoV, RVFV, WNFV, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), Foot and Mouth Disease Virus (FMDV), *Coxiella burnetii* and *Brucella spp.*

Seroprevalence of MERS-CoV, CCHFV and *Coxiella burnetii* was high among all age groups and sampling locations, with seropositivity rates of 49.8 %, 74.3 % and 50.1 %, respectively. Expectedly, seropositivity increased with increasing age of the dromedary camels, however, seropositivity of CCHFV in juvenile camels (< 24 month of age) was already high at 54.5 %. Multiple infections were common in the examined group of dromedary camels, with a seropositivity rate for infections with MERS-CoV, CCHFV and *Coxiella burnetii* as high as 70 % in one of the sampling locations.

Examining the seroprevalence of the same pathogens in the farmers and their families will reveal if those pathogens could also be transmitted to the human population in the region.

NO 05 ☣ 12+3min

Preparedness, Surveillance and Response to Crimean-Congo Hemorrhagic Fever Virus in Kosovo

K Sherifi[1], X Jakupi[2], D Hajdari[2], M Ajazaj[3], R von Possel[4], P Emmerich[4]
1- University of Prishtina Hasan Prishtina, Department of Veterinary Medicine, Pristina, UNK; 2- National Institute for Public Health, Department of Microbiology, Pristina, UNK; 3- University Clinical Centre, Clinic of Infectious Diseases, Pristina, UNK; 4- Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, DEU

In the last decades Kosovo was faced with the local outbreaks of Crimean-Congo hemorrhagic fever (CCHF) virus infections in humans. The CCHF virus belongs to the genus Orthonairovirus, to the Nairoviridae family within the Bunyavirales order and is mainly transmitted by ticks of the family Ixodidae, and also by close contact with the tissues of infected animals and humans. From the year 1995 to 2022, 244 cases of CCHF in humans have been registered, of which 63 have died (case fatality rate 25.8%), while most of the infected were farmers. The last CCHF outbreak in Kosovo was occurred in 2013, where out of 26 infected people, 11 of them ended fatally. In the same year, the government of Kosovo has established the Inter-Ministerial Committee lead by the Ministry of Health, which have launched the action plan for the preparedness and response to CCHF virus. The important activities of the action plan were also supported by the German biosecurity program. Two projects have been carried out from 2013 to 2019 'Sustainable Biosecurity for Diagnostic and Surveillance of CCHF in Kosovo', implemented by Bernard Nocht Institute for Tropical Medicine in Hamburg, whereas the Kosovar public institutions were supported with equipment, training on diagnostics and biosafety/biosecurity and the implementation of CCHF virus surveillance in ticks and animals. The outputs of the action plan and German projects were: the reduction of infected people, only 10 infected persons in 9 years (2014-2022), which one have died; the reduction of people bitten by ticks; treatment of domestic animals with acaricidal drugs to reduce the tick fauna; decreasing the CCHF virus prevalence in ticks, which out of 2857 tick tested, 19 of them were positive on CCHF viral RNA (0.66%) mainly in *Rhipicephalus bursa* (15 ticks) and *Hyalomma marginatum* (4 ticks).

NO 06 ☣ 12+3min

Strengthening sustainable biosecurity capacities across partner countries of the German Biosecurity Programme: A joint alumni network initiative by the *Global Partnership*

Initiated Biosecurity Academia for Controlling Health Threats (GIBACHT)* and the *German Online Platform for Biosecurity & Biosafety (GO4BSB)

E Mertens[1], K Aleksic-Babic[2], BM Bürkin[3], A Dolmov[4], T Habermann[1], C König[2], A Schulz[5], F Stoek[5], J Pelikan[3], C Pimmer[3], E Rutebemberwa[6], G Zikeli[7], DI Puradiredja[1]
1- Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, DEU; 2- Robert Koch Institute, Berlin, DEU; 3- Swiss Tropical and Public Health Institute, Allschwil, CHE; 4- Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, Berlin, DEU; 5- Friedrich-Loeffler-Institute (FLI), Institute of Novel and Emerging Infectious Diseases (INNT), Greifswald - Insel Riems, DEU; 6- African Field Epidemiology Network, Kampala, UGA; 7- Bundeswehr Institute of Microbiology, Munich, DEU

Since 2013, the framework of the Federal Foreign Office funded German Biosecurity Programme provides training in biosafety and biosecurity, improving the capacity of partner countries to respond to biological risks. The multilateral fellowship training programme GIBACHT has trained over 100 public health professionals from 27 partner countries, and the GO4BSB platform has supported more than 950 users from over 50 countries with digital teaching and learning content. In order to sustain biosecurity capacities and to strengthen regional networks, it is important to capitalise on the by-now considerable number of programme alumni. To this end, GIBACHT and GO4BSB have joined forces to implement a programme-wide alumni initiative. Participants who successfully completed a training by a German Biosecurity Programme project are invited to become part of the programme's alumni community and to enrol in the GO4BSB-based alumni portal. Alumni engagement activities include professional networking opportunities via GO4BSB, webinars on biosecurity related topics, and tailored initiatives such as the 'GIBACHT Regional Strengthening Initiative'. These activities positively impact interactions among all participants of the German Biosecurity Programme by providing opportunities for networking, knowledge sharing, and collaboration. Moreover, it strengthens the social capital among partner institutions in Germany and abroad, contributing to the sustained impact of the programme.

NO 07 ☣ 12+3min

Diagnostics and surveillance of class 3 and 4 risk viruses in Ukraine in the context of the German Biosecurity Programme

IV Demchyshyna[1], OA Hluzd[1], IM Korshak[1], LM Chernenko[2], IV Kuzin[3], R von Possel[4], P Emmerich[4]
1- State Institution Public Health Center of the Ministry of Health of Ukraine, Virology reference laboratory, Kyiv, UKR; 2- State Institution Public Health Center of the Ministry of Health of Ukraine, Director General, Kyiv, UKR; 3- Ministry of Health of Ukraine, Deputy Minister of the Ministry of Health of Ukraine, Kyiv, UKR; 4- Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, DEU

Due to Ukraine's geography, there is a constant risk of, firstly, the introduction of new pathogens by migratory animals and birds, and, secondly, of the emergence and reemergence of human pathogens endemic to Ukraine. A significant number of diseases that are characterized by fever of unknown origin are registered annually in the country. In Ukraine, there is a lack of rapid, modern diagnostics for the detection of some infections. In addition, the war in Ukraine has complicated diagnostic testing for fevers of unknown etiology.

Having previously identified cases of Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndrome, and West Nile fever in the territories of Ukraine, we hypothesized that these previously identified viruses, along with other emerging viruses, are responsible for fevers of unknown etiology that are as yet undiagnosed.

Between 2016 and 2022 a total of 668 serum samples were taken from patients with a history of fever of unknown etiology. We found positive samples for Hantavirus (n=25), West Nile virus (n=51), Dengue virus (n=27) and Chikungunya virus (n=5).

In order to determine the presence of CCHFV, HNTV and other viruses in regional areas of Ukraine, we also conducted a seroprevalence study with a total of 3,928 samples collected between 2016 and 2022 in 25 oblasts.

Oral Presentations on Wednesday, October 25

Audimax / 08:30 ... 10:45

O Zoonoses

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

OO 01 ☣ 25+5min
Antivirals for Flaviviruses

J Holoubek, L Eyer
Veterinary Research Institute, Brno, CZE

[pending]

OO 02 ☣ 12+3min
Indirect immunofluorescence testing of IgM against sandfly fever virus in patients with evidence of acute neurological disease

S Hohensee[1], A Hachid[2], N Bourdjoul[2], F Khardine[2], K Lamani[1], M Janku[1], S Saschenbrecker[1], E Lattwein[1]
1- Institute for Experimental Immunology, affiliated to EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, DEU; 2- Institut Pasteur d'Algérie, Laboratoire des arbovirus et virus émergents, Algiers, DZA

Introduction: Sandfly fever is a viral disease transmitted to humans through various sandfly species (*Phlebotominae*) during blood sucking. Symptomatic sandfly fever virus (SFFV) infections typically present as a self-limiting flu-like illness, with only the SFFV species Toscana (TOSV) causing neurologic symptoms. The disease is widespread in the Middle East, Central Asia, Northern Africa and the Mediterranean, but climate change is predicted to expand its distribution into Central Europe. In this study, the serodiagnostic performance of an SFFV indirect immunofluorescence assay (IFA) was evaluated in patients with confirmed or suspected SFFV infection of the central nervous system (CNS).

Methods: Samples were collected 2-24 days post symptom onset (dpso) from 31 Algerian patients whose clinical presentation was consistent with TOSV infection of the CNS (e.g., meningitis). In 15 cases, PCR was performed on cerebrospinal fluid collected 1-4 dpso, confirming infection with TOSV. Anti-TOSV IgM titers were determined using the Sandfly Fever Virus Mosaic 1 IFA (EUROIMMUN), which is based on cells separately infected with the SFFV species Sicilian, Naples, Toscana and Cyprus. Samples were tested starting from a dilution of 1:10.

Results: Anti-TOSV IgM was detected at titers of $\geq$ 1:10 in 26/31 (83.9%) of supposedly SFFV-infected patients, including 13 PCR-confirmed cases, while five were non-reactive. The highest median IgM titers ($\geq$ 1:320) were observed within two weeks after symptom onset.

Conclusion: The Sandfly Fever Virus Mosaic 1 IFA enables sensitive and specific detection of anti-SFFV (TOSV) IgM antibodies, indicating the suitability of serological testing to support the diagnosis of sandfly fever in symptomatic patients.

OO 03 ☣ 12+3min
Resurgence of Rift valley fever virus among human cases in Niger after the 2016 outbreak

M Issa, H Ousmane, S Adamou, B Aoula, B Issaka, A Lagare
Centre de Recherche Médicale et Sanitaire, Niamey, NER

Background: Rift Valley fever virus (RVFV) is a mosquito-borne arbovirus causing severe disease in humans and ruminants. The disease is endemic in most sub-Saharan African countries, with high sanitary and economic impact. The infection is generally asymptomatic in human, although fatal cases might occur in $< 3\%$. In Niger, the first documented outbreak of RVF occurred in 2016 reported 266 confirmed human cases and 32 deaths. Since the end of this episode, a surveillance system of arboviral diseases has been settled. We hereby, aim to report the detection of RVFV among human cases in early 2023.

Methods: Part of the arbovirus surveillance, serum samples were collected on suspected cases fulfilling the case definition. Samples were sent to the national reference laboratory for hemorrhagic fever virus for testing. Nucleic acid extraction was carried out with QIAGEN kit using the glove box provided in the G5 Sahel mobile laboratory plateform. RVFV detection was performed using Mic qPCR cycler. Furthermore, differential diagnostic of others arbovirus including DenV, CCHFV, YFV, ZIKV, WNV and CHIKV was conducted. Following the case confirmation, entomological surveys was conducted enabling the collection of vector mosquitoes for identification and virus detection.

Results: A total of 115 samples originating from three districts were tested between January and February 2023. Male sex represents 72.17% and the average age of all the patients was 22 years. We confirmed 5 (4.3%) cases positive for RVFV, which are mainly breeders. All the samples were negative for the differential detection of others arbovirus. From the entomological survey, 78 Aedes and Culex sp were captured and all were tested negative for RVFV and others arbovirus.

Conclusions: Recent detection of RVFV suggests the persistence of the virus in the animal reservoir. Therefore, active surveillance through One Health approach should be reinforced in order to promptly inform decision makers for effective control measures.

OO 04 ☣ 12+3min

Tick-borne encephalitis virus in ticks from Latvia

L Chitimia-Dobler[1], G Dobler[1], D Lang[1], A Bormane[2], R Ranka[3], S Schaper[1], Z Freimane[2], D Zavadska[4]
1- Bundeswehr Institute of Microbiology, Munich, DEU; 2- Centre for Disease Prevention and Control of Latvia, Infectious Diseases Surveillance and Immunization Unit Epidemiology, Riga, LVA; 3- Latvian Biomedical Research and Study Centre, Riga, LVA; 4- Department of Pediatrics, Riga Stradins University, Riga, LVA

Ticks are important parasites of economic and public health because of their ability to transmit zoonotic diseases. Tick-borne encephalitis virus (TBEV) is a Flavivirus with five main subtypes of which three, the European (TBEV-EU), the Siberian (TBEV-Sib) and the Far Eastern subtypes (TBEV-FE) are supposed to circulate in Latvia. Several hard tick species are involved in TBEV circulation and transmission in nature.

In Latvia, only few studies concerning TBEV circulation have been conducted. A large-scale country-wide study provided a snapshot of the distribution patterns of *Ixodes* and *Dermacentor* ticks in Latvia and an overview of tick-borne pathogens in Latvian field-collected ticks.

The aim of the present study was detecting, isolating, and sequencing the full genomes of the TBEV subtypes circulating in Latvian ticks. In 2019 and 2021 to 2023, ticks were collected by flagging in two Latvian regions. Ticks were morphologically identified and pooled (10 ticks/pool) and screened for TBEV RNA using a RT-qPCR. The positive pools were further investigated by sequencing the E gene and then the full genome, followed by the virus isolation. Totally, 2,421 ticks were collected, with *Ixodes ricinus* as the dominant species (2,287 specimens) followed by *Ixodes persulcatus* (130 specimens) and *Dermacentor reticulatus* (4 specimens). *Ixodes ricinus* carried TBEV-EU and *I. persulcatus* carried TBEV-Sib. In conclusion, two TBEV subtypes were detected and isolated in Latvia. Further investigations are necessary to better understand the natural transmission and the medical importance of these TBEV.

OO 05 ☣ 12+3min

Seroprevalence of anti TBE-IgG and anti-NS1-IgG in blood donors in a highly endemic TBE area in south-eastern Germany

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Tick-borne encephalitis (TBE) is the most important tick-borne viral disease in Central Europe. Since the introduction of an effective vaccine no seroprevalence studies have been possible. Therefore, no actual data on the incidence and prevalence of TBE infection in a population is currently available.

We developed an ELISA to detect IgG antibodies against NS1 antigen of TBEV indicating recent or past infection. Using this new test, we tested 1.300 sera from blood donors against TBEV IgG antibodies differentiating between vaccine-induced and infection-induced IgG antibodies.

1.300 sera were screened by a conventional TBE-IgG ELISA. Positive sera were re-tested by NS1-IgG ELISA and by TBEV neutralization test (NT) to distinguish between vaccine-induced and infection-induced antibodies. The NT was applied to exclude IgG cross reactions with other flaviviruses, e.g. yellow fever vaccination, West Nile infection or dengue infection.

The preliminary results show a TBE-IgG prevalence of 85%. Of these, 2,6% reacted positive against TBEV-NS1-IgG, indicating past infection. Most of the other TBEV-IgG positive sera reacted positive in the TBEV NT indicating past vaccination against TBE and excluding other flavivirus infections or vaccinations.

Our data indicate a much higher vaccination rate as reported by official public health data. About 2% of all blood donors and about 10% of non-vaccinated blood donors exhibit serological evidence of a past TBEV infection.

OO 06 ☣ 12+3min

Monitoring studies of ticks as carriers

of tick-borne borreliosis and encephalitis in Dnipropetrovsk Oblast from 2018 to 2022

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State Institution Dnipropetrovsk Oblast Center for Disease Control and Prevention of the Ministry of Health of Ukraine, Dnipro, UKR

Introduction: Ticks are the vector of many vector-borne diseases, including Lyme disease (LD) and tick-borne encephalitis (TBE) virus. The aim of the research was to study the extent of LD and TBE infection in ticks removed from humans and collected in the field from 2018 to 2022 in Dnipropetrovsk region, as well as to analyze infection by species.

Methods: Dark-field microscopy was used for the detection of LD-Borrelia, and ELISA and PCR were used to detect TBE antigen in ticks and TBE RNA, respectively.

Results: From 2018 to 2022, 8360 ticks were examined for LD. Of these, 2857 were positive 34.2%. The majority of ticks affected by Borrelia included the following 6 species: *Ixodes ricinus* (78.2%), *Dermacentor marginatus* (9.3%), *Dr. reticulatus* (1.3%), *Rhipicephalus rossicus* (7%), *Rh. sanguineus* (4%), *Hyallomma marginatum* (0.2%). The distribution of positive results by year was: 2018 — 29.4%, 2019 — 30.8%, 2020 — 33.1%, 2021 — 39.1%, 2022 — 40%. In addition, 263 ticks were studied by ELISA for TBE antigen. Of these, 1.5% (4 ticks — *I. ricinus*) were positive of which 2 ticks were also affected by *Borrelia*. 325 ticks (13 pools) were tested by PCR for TBE RNA, the results are negative.

Conclusions: In Dnipropetrovsk Oblast, there is an annual increase in the prevalence of *Borrelia* affected ticks. The main vector of LD and TBE is *Ix. ricinus*, which has a greater prevalence among other species. The results indicate need to improve the diagnosis of LD and TBE in health care institutions.

OO 08 ☣ 12+3min

Distribution of Bacterial Coinfection in COVID-19

S Bozoraliev
Republican Specialized Scientific and Practical Medical Center Epidemiology Microbiology Infectious and Parasitic Diseases, Tashkent, UZB

Background: Microbial coinfection raises the chance of illness severity in people with SARS-CoV-2 infection. The prevalence of bacterial coinfection in hospitalized patients with confirmed SARS-CoV-2 infection was determined in this study.

Methods: 672 laboratory-confirmed SARS-CoV-2 patients, who underwent stationary treatment at the RSSPMCEMIPD from 2020 to 2022 were studied. Secondary bacterial infections were identified by sowing sputum

Results: The most prevalent bacteria were found to be *K. pneumoniae* and *S. aureus*. Except for *E. faecalis* and *A. baumannii*, six pathogenic bacteria species were found in people aged 18 to 44. All 8 types of pathogens, including *E. faecalis* and *A. baumannii*, were detected in older patients. The frequency of occurrence of pathogenic bacteria in age groups did not differ significantly. The causative agents of pathogenic bacterial coinfection were as follows: *K. pneumoniae* (110, 16.4%), *S. aureus* (82, 12.2%), *E. coli* (38, 5.7%), *S. pneumoniae* (14, 2.1%), *S. pyogenes* (12, 1.8%), *P. aeruginosa* (5, 0.7%), *E. faecalis* (2, 0.3%) and *A. baumanii* (1, 0.1%).

Conclusions: Despite the relatively high rate of substantiated microbial coinfection in confirmed SARS-CoV-2 patients in our investigation, no significant association was reported between coinfection and severity, mortality, or other indicators. Late secondary bacterial infections are less prevalent, and more study is needed to determine their occurrence, nature, and impact.

OO 09 ☣ 12+3min

SARS-CoV-2 seroprevalence after the first epidemic wave, 2020 in Bamako, Mali

B Kouriba[1], M Cissoko[2], MK Bediane[3], AK Sangaré[1], J Landier[4], A Katile[2], I Berthé[5], I Théra[2], J Gaudart[3], I Sagara[2]
1- Centre d'Infectiologie Charles Merieux-Mali, Bamako, MLI; 2- Malaria Research and Training Center, Bamako, MLI; 3- Aix Marseille Univ, IRD, INSERM, SESSTIM, Marseille, FRA; 4- Institut de recherche pour le développement, Marseille, FRA; 5- Direction Générale de la Santé et l'Hygiène Publique, Bamako, MLI

Mali, like many countries in sub-Saharan Africa, has reported only a limited number of COVID-19 cases. This study assesses the proportion of the population that has been infected with SARS-CoV-2 in Bamako and compares it to reported data to shed light on the COVID-19 epidemic in Mali.

The study took place in 3 districts of commune VI of Bamako. Participants were enrolled at home, answered a questionnaire, and provided a blood sample. IgM and IgG antibodies against the Spike protein were measured by ELISA. The seroprevalence and the 95% confidence interval were calculated for commune VI, by district, by age group.

From September 16 to 29, 2020, 1526 participants

were included, and 1367 valid serology results obtained. Crude seroprevalence was 16.5% (225/1367, 95% CI = 14.5–18.5%). The prevalence was similar between the 3 neighborhoods (*Chi2, p = 0.91*). Considering the distribution by age and sex of the sample compared to that of the population, the prevalence in the general population was estimated at 15.8%, or more than 380,000 infections. The expected number of deaths related to COVID-19 would be less than 1,500 between March and September.

This cross-sectional study suggests a slow but widespread of SARS-CoV-2 during the first 6 months of the pandemic in Bamako. The low reported mortality does not reflect the expected deaths, but the estimated lethality remains low (< 4 deaths/1000 cases), calling for study to understand better this low lethality.

Audimax / 11:00 ... 12:00

P

Poster Awards and Farewell

Chairs: Roman Wölfel (DEU) and Joachim J. Bugert (DEU)

Poster Awards Ceremony

Closing Remarks

R Wölfel
Conference Chair and Director, Bundeswehr Institute of Microbiology, Munich, DEU

Poster Presentations

Poster presenters should be at their poster between 1:30 to 3:30 p.m. on Tuesday!

Foyer

Poster

Chair: Katrin Zwirglmeier (DEU)

BP 07

Nanomedical Approach for Treating COVID-19: An *In-Vitro* PoC Study

FIL Hucke[1], F Schwarze[1], N Bayer[1], MK Özçürümez[2], R Wölfel[1], A Canbay[2], JJ Bugert[1], RK Gieseler[2]
1- Bundeswehr Institute of Microbiology, Munich, DEU; 2- University Hospital Knappschaftskrankenhaus, Department of Medicine, Bochum, DEU

Introduction / Materials and methods: We evaluated lipid nanocarriers (NCs) loaded with the antiviral lectin griffithsin (GRFT) for interference with SARS-CoV-2 in infected Vero E6 cells. Under infected [IMB muc 1 (original virus); IMB CB — B1.1.7 (α-variant)] or non-infected conditions, soluble GRFT, NC-encapsulated GRFT + free GRFT (NC-G1) or NC-encapsulated GRFT only (NC-G2) were employed vs negative controls or remdesivir (R).

Results: 1. Cell viability assays (MTS) with antivirals at 0.5 μM: NC-G2 was the most effective formulation with 55% cell survival in the presence of antivirals for the α-variant (32.9% cell survival vs original; $p \leq 0.0001$) compared to untreated control (0% survival) and was not cytotoxic (cell viability: 85.23% $\pm$ 1.54% and 89.09% $\pm$ 3.33%; $n = 2$). R was not active at 0.5 μM (IC50 of 11.97 μM in MTS cell viability assays). 2. Viral release into the supernatant over 2 days: Viral RNA was quantitated by qRT-PCR. IC50 values were 120 pM (NC-G2), 10.29 nM (GRFT), and 15.5 nM (R), respectively. We thus demonstrate SARS-CoV-2 inhibition with NC-G2 at 1/86th of the concentration of soluble GRFT and a 130-fold advantage over R.

Discussion: Given that GRFT is highly tolerable and that low-cost, large-scale production of purified recombinant GRFT is feasible, pharmaceutical-grade NC-G2 may be developed for treating COVID-19 and, potentially, other emerging coronavirus infections. We recently presented a spray-dried formulation for pulmonary delivery of antibiotic-loaded NC (Thiyagarajan et al., 2021) that might be adapted accordingly for intervention in early-stage COVID-19.

BP 08

Molecular effects of co-administering of artemisinin antimalarials with aspirin or vitamin C

V Pashynska
B. Verkin Institute for Low Temperature Physics and Engineering of the NAS of Ukraine, Molecular Biophysics Department, Kharkiv, UKR

Currently artemisinin-based combination therapy is recommended by the WHO as a first-line treatment of falciparum malaria. Molecular effects caused by artemisinin antimalarials co-administering with other structurally different drugs can induce the modulation of the drugs activity. Thus, the data about intermolecular interactions of the antimalarials with biomolecules and other medicines are particularly important for the medical practice, in particular, in the case of co-administering of the artemisinin-type drugs with some anti-inflammatory or antioxidant agents. The presented combined model study by electrospray ionization mass spectrometry and DFT quantum chemical calculations was devoted to *in vitro* examining and structure & energetic characterization of the intermolecular interactions of artemisinin, dihydroartemisinin, α-arthemether, arthesunate with molecules of acetylsalicylic acid (aspirin) or ascorbic acid (vitamin C). The results revealed a competition between the antimalarial drugs and aspirin (or vitamin C) for stable noncovalent association with molecules of membrane phospholipid dipalmitoylphosphatidylcholine. Formation of pair stable complexes between molecules of the antimalarials and acetylsalicylic (or ascorbic) acids was also demonstrated. The observed phenomena testify to the potential modulation of artemisin-type drugs' activity already at the stage of transmembrane transfer under their co-administering with aspirin (or vitamin C).

BP 09

Dogs and horses as sentinels of flavivirus circulation in south-eastern France

Y Laidoudi[1], G Durand[2], S Watier-Grillot[3],

G Grard[2], B Davoust[4]
1- Aix Marseille Univ, IRD, AP-HM, MEPHI - IHU Méditerranée Infection, Marseille, FRA; 2- National reference centre for Arboviruses, French armed forces biomedical research institute (IRBA), Unité des virus émergents, Aix Marseille Univ, IRD 190, INSERM 1207, Marseille University Hospitals (AP-HM), Marseille, FRA; 3- French military health service, Animal epidemiology expert group, Tours, FRA; 4- Aix Marseille Univ, IRD, AP-HM, MEPHI - IHU Méditerranée Infection, French military health service, Animal epidemiology expert group, Marseille, FRA

West Nile virus (WNV) is a mosquito-borne Flavivirus, inducing meningoencephalitis in humans and horses. In the southeast of France, in 2022, there were five equine and four human cases of WNV and two bird cases of Usutu virus (USUV). The first human case in France of USUV was diagnosed in 2016 in Montpellier. The aim of our study was to evaluate the seroprevalence of WNV and USUV in dogs and horses from south-eastern France. In the Var, Bouches-du-Rhône and Hérault departments, blood samples were taken from 310 dogs and 148 horses, in 2021-2022. Each serum sample was tested for IgG against WNV and USUV by using an in-house ELISA. Microneutralization tests (SNT) against WNV, USUV, tick-borne encephalitis virus (TBEV) and Bagaza virus (BAGV) were used to confirm the specificity of the ELISA IgG-positive and doubtful sera. A total of 17.4% of dogs and 4% of horses scored positive on the ELISA. Overall, 14, 4, and 7 dogs were confirmed positive on the SNT for WNV, USUV and doubtful, respectively. Only one horse was detected by WNV SNT and one doubtful. Interestingly, a non-negligible seroprevalence of non-WNV, non-USUV, non-TBEV and non-BAGV infections was detected with ELISA in 18.6% (28/150) and 3.7% (4/106) of the dogs and horses, respectively from the Hérault department. However, new investigations are required to characterize the as yet unidentified flaviviruses in this area.

BP 10

Prevalence of COVID-19-associated pulmonary aspergillosis and antifungal drugs resistance of *Aspergillus* spp. isolated from COVID-19 patients in Uzbekistan

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Background: Morbidity and mortality from COVID-19-associated pulmonary aspergillosis (CAPA) remain high. The aim of the study was to determine CAPA prevalence and antifungal resistance of *Aspergillus* spp. isolated from COVID-19 patients in Uzbekistan.

Methods: Fifty COVID-19 patients from the intensive care unit were enrolled. Serum *Aspergillus* IgG levels were detected by ELISA. *Aspergillus* spp. were identified and drug resistance of isolates were determined by mycological method and E-test, respectively.

Results: *Aspergillus* IgG were detected in 14 (28%) of COVID-19 patients. *Aspergillus* spp. were isolated from sputum of 9 (18%) patients. But, only 11 (22%) patients met the diagnostic criteria for CAPA. The mortality rate of CAPA patients was 45.4%. *Aspergillus fumigatus* was the most frequently isolated species (55.5%) followed by *A. niger* (33.3%) and *A. flavus* (11.1%). 100% of *Aspergillus* spp. isolates were susceptible to amphotericin B. The susceptibility rate of *A. fumigatus* to itraconazole and voriconazole was 60 and 80%, respectively. 100% of *A. niger* and *A. flavus* isolates were susceptible to antifungals.

Conclusions: Our study reveals a high mortality and morbidity of CAPA in Uzbekistan. The *in vitro* activity of amphotericin B, voriconazole and itraconazole indicates their importance in the therapy of aspergillosis.

BP 11

A Randomised Prospective Trial Comparing Rectal Culture-Based Versus Empirical Antibiotic Prophylaxis for preventing Infectious Complications following Transrectal Prostate Biopsy

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The aim of this study is to evaluate the benefit of targeted antibiotic prophylaxis (TAP) based on rectal swab culture in comparison with standard empiric antimicrobial prophylaxis in patients undergoing transrectal ultrasound-guided needle biopsy of the prostate (TRUS-BP) in terms of infectious complications. Prospective study that randomized 157 patients proposed for PB into two groups. Group 1 (G1): Empirical antibiotic prophylaxis with ciprofloxacin. Group 2(G2): TAP according

to rectal swab performed 10 days before PB. The prevalence of digestive carriage of Fluoroquinolone-resistant Enterobacteria (FQRE) and risk factors for carriage of FQRE were studied. The incidence of infectious complications after PB in each group was compared. G2 included 80 patients versus 77 in G1. There was no difference between the two groups regarding age, diabetes, prostate volume, PSA, number of biopsy cores, and risk factors for FQRE. In G2, the prevalence of digestive carriage of FQRE was 56.3%. In the case of digestive carriage of FQRE, TAP according to the rectal swab culture with 3rd generation cephalosporins was performed in 73.3%. Patients with FQRE had a history of use of FQs within the last 6 months in 17.8% (p=0.03). The rate of infectious complications after PB was 3.8% in G1 and 10% in G2 (p = 0.02). The prevalence of FQs resistance in the intestinal flora in our population was high. The risk factor for resistance was the use of FQs within the last 6 months. TAP adapted to rectal swab, mainly with 3rd generation cephalosporins, significantly reduced the rate of infectious complications after PB.

BP 12

Heterocellular spheroids as a new model for antivirals testing at the blood-brain-barrier (BBB)

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We aim to create heterocellular neurovascular spheroids to simulate viral infection at the BBB. This will allow us to monitor the time it takes the virus to infect internal cells of the spheroid and the effect of antivirals in this process.

Material and methods: Infection rates of endothelial (HBEC-5i) or glioblastoma (U138) cell lines with EGFP based reporter viruses are monitored in single or multiple-cell cultures and in 2D or 3D growth environments. The detection of VE-Cadherin and Claudin 5 at the border of the endothelial cells and glial cells is used as an indicator of a functional BBB. The progression of viral infection in the presence and absence of antivirals β-d-N 4-Hydroxycytidine (NHC) and tecuvirimat (TPOXX) is monitored by detection of reporter activity in live and inactivated spheroid cells.

Results: Preliminary results show that 3D grown mixtures of endothelial and glioblastoma cell lines form heterocellular spheroids and are viable for at least 48 h. Heterocellular neurovascular spheroids can be infected with EGFP reporter viruses and virus propagation starts in endothelial cells.

Conclusion: We show for the first time infection of heterocellular neurovascular spheroids with reporterviruses and their suitability to assess the effect of antivirals in this process.

BP 13

Safety study of poly-phosphoesters as carriers for targeted antibiotic delivery

M Kozak[1], D Ostapiv[1], I Petruh[1], Y Bodnar[1], N Kuzmina[1], A Stasiuk [2], V Samaryk[2], V Vlizlo[3]
1- Institute of Animal Biology of NAAS of Ukraine, Laboratory of molecular biology and clinical biochemistry, Lviv, UKR; 2- Lviv Polytechnic National University, Department of Organic Chemistry, Lviv, UKR; 3- S. Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Department of Internal Animal Diseases and Clinical Diagnostics, Lviv, UKR

Antibiotics combined with a drug delivery agents could reduce the emergence of antibiotic resistant microorganisms and also provide desirable therapeutic effects with reduced toxicity. The aim of this study was to assess the safety of poly(phosphoester)s as antibiotic delivery agents and assess their effectiveness against microorganisms. Synthesis of the poly(phosphoester)s was performed via polycondensation, using PEG-400 or PEG-600 to obtain two kinds of polyesters (P4 and P6) with different molecular weights. Animal studies were performed in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). Balb/c mice were intramuscularly injected with poly(phosphoester)s and their complexes with antibiotics (doxycycline, oxytetracycline and amoxicillin). Blood biochemical analysis and histology were used to assess toxicity. Antimicrobial studies were performed on *E. coli, S. aureus* and *P. aeruginosa* laboratory strains. Antibiotic complexes with P4 and P6 effectively inhibited bacterial growth compared to their commercial forms. Biochemical analysis indicates that the P6 polymeric carrier had a toxic effect on the liver (AST activity increased by 44% and ALT activity decreased compared to control and exited normal physiological parameters). P4 did not induce any statistically significant changes in these parameters. Histology did not show any pathological changes. As such, the P4 poly(phosphoester) complexes with antibiotics may serve as effective antibiotic carriers for antimicrobial treatment in the future. Research will be continued to confirm the obtained results *in vivo* and *in vitro* on field strains of microorganisms.

BP 14

Tunisian National Action Plan for Antimicrobial Resistance Surveillance

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For the past two decades, Tunisia has faced an overall increase in microbial resistance. In human health, antimicrobial resistance surveillance showed that resistance of *Escherichia coli* to 3^{rd} generation cephalosporins increased from 4% in 2004 to 18.8% in 2019, while resistance of *Klebsiella pneumoniae* to carbapenems increased from zero before 2004 to 22.4% in 2019. In 2019, Tunisia developed its National Action Plan (NAP) on AMR, mainly addressing awareness, surveillance, infection prevention and control, and rational use of antibiotics. The present study relates the progress made in the establishment of the national antimicrobial resistance surveillance network and monitoring system, lab upgrades, and strengthening of the quality control system. Today, the Tunisian AMR network is composed of 12 surveillance university hospital that work in coordination with the National Reference Lab and the National Coordinating Center. Many actions have been carried out (several training series, Lab assessments), and corrective measures on quality, biosafety, and biosecurity have been implemented in the different labs.

Currently, surveillance is based on bacterial data, and we work on the integration of clinical and epidemiological data and the integration of the private sector first and the animal sector second. However, looking for the best IT solution is a must for the next period to ensure network fluidity, real-time data sharing, and feedback to all stakeholders.

BP 15

European multi-centre study to establish MIC and zone diameter epidemiological cut-off (ECOFF) values for *Brucella melitensis*

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Background: *Brucella* (*B.*) *melitensis*, the causative agent of brucellosis, is a zoonotic agent causing about 500,000 human cases annually and is endemic in the Mediterranean basin, the Middle East, parts of Central and South America, Africa and Asia. The disease is associated with a high risk of chronification and relapses and requires a long-term antimicrobial combination therapy. Until now, antibiotic resistance has been rare. However, mutations associated with antibiotic resistance have been reported, e.g. in the *rpoB*, leading to phenotypic resistance towards rifampicin. Antimicrobial susceptibility testing (AST) standards for *B. melitensis* are not available and it is not yet listed in the EUCAST breakpoint tables. In this study, we aimed to establish minimal inhibitory concentration (MIC) and zone diameter distributions of wild-type (WT) strains from different origins to set epidemiological cut-off (ECOFF) values for *B. melitensis* using EUCAST methodology.

Materials and methods: Under the framework of an EU-funded Joint action 499 *B. melitensis* isolates (20 to 247 per centre) from human and animal origin were tested at 6 study sites against 9 antimicrobials by disc diffusion (DD) method and broth microdilution (BMD). Each centre validated the methods with three QC strains (*E. coli* ATCC 25922, *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213) and compared results with EUCAST QC tables. BMD was performed according to ISO 20776-1, but with prolonged incubation as described for *B. melitensis* before (Tscherne *et al.* 2022). DD was performed with EUCAST Mueller-Hinton agar for fastidious organisms. *B. melitensis* MIC distributions were

submitted to EUCAST and aggregated results were curated in accordance with EUCAST SOP10.2.

Results: For each drug investigated, ECOFF values were defined based on 249 to 499 observations. MIC distributions, revealed a wild-type phenotype encompassing the majority of the isolates. Single strains showed slightly increased MICs, above the ECOFFs for 5 of the tested substances. If they have acquired resistance mechanisms will be further analysed genotypically.

Conclusions: In this multi-centre study, we have validated the use of BMD and DD methodology for AST of *B. melitensis* and determined MIC and zone diameter ECOFFs. The ECOFFs can now be used to distinguish between WT and non-WT organisms. Together with clinical data they will serve as background data to set clinical MIC breakpoints and disc diffusion correlates for *B. melitensis*.

BP 16

Molecular basis of antimicrobial properties of green nanocoating effective against virus and bacteria

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Introduction: Microbial colonization of surfaces constitutes a dangerous reservoir of pathogens that contribute to spread of infections with repercussions on human health and a heavy economic burden. The present project aims to analyze the antimicrobial properties of an innovative self-disinfecting nanocoating based on copper nanoparticles doped with natural peptides and of good environmental safety. In particular was investigated antimicrobial properties on *E. Coli* and SARS-CoV-2 and evaluated early molecular mechanisms in response at different exposure time and concentrations.

Materials and methods: Standard procedures to evaluate minimum inhibition doses to distinct substrate were applied. After early exposure to the nanocoating, total RNA was extracted and libraries for transcriptome investigations (RNA-Seq) were prepared. Two NGS platforms, Illumina and MGI, were used. For the bioinformatic analysis an ad hoc pipeline was implemented to analyze the sequencing output of each sequencing technology.

Results: Different concentrations of eugenol, thymol and menthol were used to treat distinct microorganism. Our results indicate that substrates can inhibit the microorganism growth and cause an extensive transcriptome response. In bacteria Reactive Oxygen Species induce a large number of genes related to cell damage, cell membrane repair system, and DNA repair system and genome damages in viruses.

Acknowledgments: This work was funded from RELIANCE project (HE programme).

BP 17

Expression of the broad spectrum antiviral Griffithsin in human cells: looking for a selective window

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At the Bundeswehr Institute of Microbiology (IMB) the lectin Griffithsin (GRFT), derived from the red algae Griffithsia, is being investigated with regard to its antiviral effect in eukaryotic cells as part of the STAN project 59-2016-STAN Therapy of Biodefense-Relevant Pathogens.

In vitro and in animal models, GRFT is antivirally active against various enveloped viruses by binding glycans with a high mannose content and thus acting as a broad-spectrum virus inhibitor [1,2]. The following three questions will be investigated in the project: a. is GRFT toxic in eukaryotic cells - induction in the Tet-On system, and determination of the cytotoxic concentration 50 (CC50) b. is GRFT expressed in eukaryotic cells antivirally active - infection of GRFT expressing cells with orthopox viruses to determine the inhibitory concentration (IC50) and the therapeutic window. c. which viruses are inhibited by intracellularly expressed GRFT.

Our approach is to have GRFT expressed by the eukaryotic cells themselves. To this end, a pLenti CMVtight eGFP Neo (w784-1) plasmid with GRFT insert under the control of a doxycycline inducible promoter will be constructed and cloned into competent *E. coli* (XL10). The plasmid and controls will be transfected into human HEK293 cells, neomycin will be used to select the transfected cells and eGFP will be used to screen for successful transfection. GRFT will be induced using doxycycline in the Tet-On system. Possible toxicity will be quantified using cell viability assays. Finally, HEK293 will be infected with orthopoxviruses at therapeutic GRFT induction levels and cell survival will be redetermined. If there is an antiviral effect and a therapeutic window, cells susceptible to other eukaryotic viruses will be transduced, and tested the same way. Results will be presented and discussed.

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BP 18

Antimicrobial resistance profile of clinical *Brucella melitensis* strains isolated in Tunisia

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Brucellosis is a serious zoonotic disease and is endemic in Mediterranean countries. In Tunisia, death cases from brucellosis are rare, occurring in no more than 2% of all cases. Generally, the antibiotics doxycyclin and rifampin are recommended in combination for a minimum of 6-8 weeks. Furthermore, while susceptibility to antibiotics testing of *Brucella* is a meticulous technique and requires strict compliance with biosafety rules, it is not applied in any laboratory. Minimum inhibitory concentrations (MICs) of 10 antibiotics (rifampin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, chloramphenicol, trimethoprim, sulfamethoxazole, gentamicin, and streptomycin) were determined using microdilution method for 36 non duplicated *Brucella melitensis* clinical strains isolated in Tunisia. Interpretation was performed according to CLSI recommendations. Fourteen strains were susceptible to all antibiotics tested and only one strain was multidrug resistant. Twenty one strains were intermediate resistant to rifampin (MICs=2mg/l), one strain was resistant to doxycycline (MIC=8mg/l), two strains were resistant to chloramphenicol and one to ciprofloxacin. In addition, all isolates were subjected to whole genome sequencing (WGS) and compared with 1000 *Brucella* genomes in a cgMLST scheme based on 2678 genes. The presence of antimicrobial resistance genes was also analyzed. The latter finding highlights the importance of the WGS data gaps to allow molecular-epidemiological investigations to determine the resistome of *Brucella* strains.

BP 19

Determining a human clinical dose for the development of Brincidofovir for Smallpox disease under the FDA Animal Rule

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Background: Despite eradication of smallpox, concerns remain about its potential use as a bioweapon. Brincidofovir (BCV) is an FDA-approved oral lipid conjugate of cidofovir with potent *in vitro* activity against *Variola* virus, the causative agent for smallpox.

Methods: In accordance with FDA's Animal Rule, two well-characterised animal models, rabbitpox and mousepox, demonstrated clinical efficacy of BCV. These are closely related to smallpox, with similar genomic and clinical characteristics. The primary endpoint was survival. Effective human doses were modelled and simulated from therapeutic exposures to BCV in animal models. Circulating BCV and peripheral blood mononuclear cell (PBMC) concentrations of the active metabolite, cidofovir diphosphate (CDV-PP), confirmed adequate exposure over a 2-week treatment course in humans.

Results: Results showed statistically significant improved survival in the animal models in all BCV arms versus placebo, even when started beyond the midpoint of disease progression. In modelling BCV and CDV-PP maximum concentrations (C_{max}) and area under the curve (AUC) in healthy humans, 200 mg weekly provided exposures to BCV and CDV-PP in excess of efficacious levels in rabbits.

Conclusions: Efficacy studies in rabbitpox and mousepox models demonstrated improved survival with BCV versus placebo. Human dose modelling demonstrated positive ratios of C_{max} and AUC in healthy humans to those in healthy and infected rabbits. Both contributed to FDA approval of BCV.

BP 20

European multi-center study to establish MIC and zone diameter epidemiological cut-off (ECOFF) values for *Bacillus anthracis*

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Background: *Bacillus anthracis*, the etiologic agent of anthrax, is a zoonotic microorganism that mostly affects herbivorous mammals, but can be transmitted to humans by contact with infected animal or their products. It is endemic almost worldwide, and it is considered a category A bioterrorism agent. If not promptly treated, *B. anthracis* infection can progress rapidly and has a high mortality rate, underpinning the importance of a timely and effective antimicrobial treatment. By now, for this microorganism, no antimicrobial susceptibility testing (AST) standards are available. Therefore, in this study, we aimed at setting up, in collaboration with EUCAST, epidemiological cut-off (ECOFF) values to distinguish between wild-type (WT) or not microorganisms.

Materials and methods: Under the framework of an EU-funded Joint action 335 *B. anthracis* isolates (17 to 146 per center) from human, environmental and animal origin were tested at 6 study sites against 10 therapy relevant antimicrobials by means of disc diffusion (DD) method and broth microdilution (BMD). Each center validated the methods testing 3 quality control (QC) strains (*E. coli* ATCC 25922, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) over 10 days and comparing the results with the EUCAST QC tables. AST was performed according to EUCAST, but with reduced incubation time compared to ISO 20776-1. *B. anthracis* MIC and DD distributions were submitted to EUCAST and aggregated results were curated in accordance with EUCAST SOP10.2.

Results: For each drug investigated, ECOFF values were defined based on 330 to 335 observations. DD and BMD data distributions, revealed a WT phenotype for the majority of the isolates. Three strains with benzylpenicillin MIC values of 32 mg/L were found, indicating resistance to this drug. MIC values above the defined ECOFF values were observed in a few strains, indicating the presence of low level resistance towards 6 antimicrobials. The genetic background to the resistance mechanisms and the phenotypic shifts observed remain to be investigated.

Conclusions: In this multi-centre study, we validated the use of BMD and DD methodologies for AST of *B. anthracis* and determined MIC and zone diameter ECOFFs for 10 antimicrobial agents. The ECOFFs can now be used to distinguish between WT and non-WT *B. anthracis*. Together with clinical data our results will pave the way for EUCAST to determine clinical MIC breakpoints for this microbial target.

BP 21

Vanguard Biodefense - A Sense, Characterize, Develop and Deliver framework

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Group is a Public Benefit Corporation that is a diversified, sustainable commercial company providing Defense and Health Agencies worldwide with high volumes of prepaid drugs and precision diagnostic tests to meet current healthcare needs and emerging biological threats. Vanguard employs its proprietary 'Sense, Characterize, Develop and Deliver' framework that enables countries to meet non-communicable disease and infectious diseases as well as to detect and immediately respond to emerging infectious disease threats, in the form of either a potential pandemic or bioweapons attack. With its global point of care, laboratory, and sequencing capabilities in key clinical environments, Vanguard has the ability to pivot virtually overnight from its steady-state disease diagnostic, therapeutic, and research operations to 24/7 emergency medical countermeasure ("MCM") response. In this emergency mode, Vanguard rapidly characterizes pathogens and then leverages its own internal research operations at Texas Biomedical Research Institute to deploy pre-evaluated FDA/EMA approved drugs (previously screened against all known pathogen types) for efficacy against the new threat. After identifying the most effective drugs, Vanguard immediately starts to manufacture either generic versions or branded drugs under a pre-negotiated license. Within 7 days of Vanguard's pivoting to an emergency response mode, its prepaid customers begin to receive millions of doses of drugs in ready-to-use formulations, along with tens of millions of point-of-care rapid diagnostic tests and lab-based test materials. These MCMs supplement other interventions (e.g., non-pharmaceutical) that are key to containing an infectious disease outbreak for any Vanguard participating country, regardless of size or wealth.

CP 06

Bioluminescence of *Vibrio fischeri*: Application for the Quantification of Neurotoxins and Heavy Metals

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Neurotoxins and environmental heavy metals create serious public health problems worldwide. However, reliable assays for the detection of these agents are sparse and difficult to perform. In order to fill this gap, we refined bioluminescence assays (BAs) based on *Vibrio fischeri* by optimizing the culture conditions and thereby enhance both the intensity and stability of the luminescence emitted by the bacteria. On one hand, the neurotoxic Paralytic Shellfish Poison (PSP) was quantified in bivalves by BAs and compared to the widely used mouse bioassay (MB) and Liquid chromatography-mass spectrometry (LC-MS). While our data suggested a significant difference between the results evaluated by LC-MS and the MB, the concentrations of the neurotoxin determined by our BAs directly correlated with the values obtained by LC-MS. On the other hand, the capability of the BA was evaluated for the rapid detection of heavy metals. Here, the detoxification of $HgCl_2$in a fluidized bed reactor by *Escherichia coli* could be readily monitored by the method. Additionally, from a panel of different metal ions, the optimized assay based on *Vibrio fischeri* correctly identified mercury as the most toxic agent. In conclusion, we could demonstrate in proof-of-concept processes how the refined BAs could be eligible for evaluating neurotoxins in surveillance programs as well as heavy metals in the environment. At the same time, the method remains simple, rapid, reliable, sensitive and cost-effective.

CP 07

An improved workflow for the sensitive detection and quantification of ricin

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Ricin is a highly toxic protein produced by the seeds of the castor bean plant *Ricinus communis*. It belongs to the family of type II ribosome-inactivating proteins and causes cell death via blocking protein synthesis by depurinating a specific adenosine from 28S rRNA. Due to its high toxicity and the fact that castor beans are widely cultivated plants and ricin extraction from castor beans is relatively easy, it is considered a potential bioterrorist agent. Therefore, a method for sensitive and reliable detection and quantification of the active toxin from various types of sample matrices is needed.

Although numerous methods have been reported to detect ricin protein, these assays generally do not distinguish between inactive and active toxin. The confirmatory method is a workflow that combines antibody capture with magnetic beads, an *in vitro* activity assay that analyses depurination of a synthetic RNA substrate, and a structural assay in which the enriched ricin is tryptically digested and peptides unique to ricin are detected and quantified by mass spectrometry.

In the workflow described here, ricin is captured and enriched from various sample matrices such as buffer, milk, or serum using asialofetuin-terminated magnetic beads. After extraction, ricin is eluted and the toxin activity is determined in a cytotoxicity assay using HeLa cells.

For the detection of depurination events in HeLa cells, we further developed a ddPCR previously described by Lewis *et al.*. This assay is based on a dual amplicon format in which one primer set is used to measure the total amount of rRNA and the second primer set amplifies the depurination site. We redesigned the reverse primer and probe for depurination site detection and changed the format from a two-step protocol, in which cDNA synthesis and PCR are performed separately, to a one-step protocol. We also adapted the procedure of the cytotoxicity assay: To cover a wide range of concentrations for ricin detection, cells were seeded at three different densities: 10^2, 10^3, and 10^4 cells per well. After treatment of HeLa cells with 10 nM ricin, the ddPCR detected depurination of 4% (10^4 cells per well) and 31% (10^2 cells per well) after 1 hour, respectively. The degree of depurination increased with time until, after 8 hours of intoxication, a depurination level of 70-80% was reached. With the here described improved workflow a limit of detection of 0.25 pM (= 0.25 fmol/mL) was achieved for the detection of active ricin.

CP 08

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

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Biotoxins are causative agents of food poisoning, but also have a history as warfare agents and could be used in a bioterrorism context. Based on several

biotoxin incidents observed in recent years, there is a need further improvement in analytical procedures to detect and identify different biotoxins.

EuroBioTox was a Horizon 2020 project integrating 63 network partners from 23 countries from the health, food and security sectors running from 2017 to 2023 (1). Under EuroBioTox, progress beyond the state-of-the-art was achieved by the production of five biotoxin certified reference materials which were released in 2022/2023. The core consortium refined measurement procedures and provided improved analytical tools, reagents and standard operating procedures within the network via a dedicated repository. 19 different training courses tailored to different biotoxins were implemented, and several e-learning courses were established. The spreading of good analytical practices was demonstrated by a series of eleven proficiency tests on biotoxins. Addressing the needs of first responders guidelines on sampling, detection and decontamination were established. Finally, five animal replacement methods for biotoxin detection were evaluated and highly relevant conclusions on more sensitive *in vitro* tests were obtained.

In summary, EuroBioTox successfully contributed to improve preparedness and response planning at national and international level by sound capacity building for biotoxins.

CP 09

Establishing of the multiplex PCR method for detection of *Clostridium botulinum*

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Clostridium botulinum is a spore-forming bacterium which is able to produce botulinum neurotoxins (BoNTs), the causative agents of botulism. BoNTs can be divided in seven antigenic types (A-G), and human cases are caused primarily by type A, B, E & F. Georgia, having a tradition of home canning, is one of the most affected countries by foodborne botulism. Since 2015, 47 non-fatal cases have been reported, mainly caused by canned vegetables. So far, routine diagnostic is based on isolation, cultivation and confirmation of BoNTs by mouse bioassay, which is still the gold standard. However, this method is labor-intensive and slow. Quantitative real-time PCR (qPCR), with its high specificity and short duration, is an essential method in clinical diagnostics. Our aim is to broaden the diagnostics of BoNTs by introducing two duplex qPCR assays (A/B & E/F). Therefore, bacteriology was performed on 61 *C. botulinum* samples requested from the repository of the NCDC. After Gram staining, DNA of these strains was tested by qPCR. In ongoing work the validation of the procedure for relevant matrices will be carried, to perform qPCR testing directly from sample material (i.e. canned vegetables). After validation, this qPCRs will be included to the national diagnostic algorithm along with serological and bacteriological tests. Overall, this achievement is of great importance for early diagnosis and epidemiological surveillance. This work is part of the German Biosecurity Programme.

DP 08

Complex synergy and training in the biological risk of operators in the migration phenomenon

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The health system of the Sicily Region, for the health management of the irregular migratory flows that insisted on the island of Lampedusa, has adopted a Migrant Health Contingency Plan for the protection of public health created in collaboration with the Ministry of Health, the WHO and implemented by Frontex in 2018. Focus of the Plan, to create a predictive system for biological defense through training and exercises.

Materials and methods: It has allowed the management of over 700000 migrants from an epidemiological point of view. An entire chapter of the aforementioned Plan was dedicated to biocontainment procedures. This resulted in a resilient regional health system which led in 2019, in the pre-pandemic period, to the creation of a training course for all those who, for various reasons, are involved in the management of patients with potential diffusive infectious diseases according to the provisions of the WHO 2005 RSI. A format called Global Biohazard Management GBM certified ISO 9001 was created in collaboration with the Ministry of Health, the Air Force and the Order of Doctors of Palermo. Increased attention was paid to dressing and undressing procedures with personal protective equipment against biohazards through an interactive audio-video system.

Conclusions: To date, over 17000 health professionals (doctors and nurses) have been trained in e-learning mode and over 1500 subjects belonging to the State Police and the Carabinieri in residential mode. GBM is equipped with dedicated APPs

which allow, in the event of events involving a large number of operators, the possibility of using all the validated and standardized procedures in real time and on a global scale.

DP 10

An overview of the Portuguese laboratory response to Mpox epidemic

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Mpox is a zoonotic disease caused by the mpox virus (MPXV). Since May 2022, several cases of mpox have been reported in different countries where the disease is not endemic. Until May 2023, 87.529 confirmed cases, including 141 deaths, have been reported by the World Health Organization in 111 countries. The aim of this study is to describe the laboratory results of mpox cases in the reference laboratory in Portugal.

Due to its accuracy and sensitivity, the laboratory diagnosis of mpox was based on the Real Time PCR method. The recommended specimens for laboratory confirmation were a lesion and an oropharyngeal swabs per case. Based on the patient's clinical presentation, other samples were considered for investigation, such as genital and/or rectal swabs, urine, and semen.

Portugal was one of the first countries to confirm mpox cases, being reported the first case on 17^{th} May 2022. To date, 953 cases have been laboratory confirmed and with the exception of nine cases (1%) in females (two of them, pregnant women), the positive cases were male (n=944; 99%), mostly in the 30-39 age group (n=420; 44.1%) and mainly men who have sex with men.

Positive cases were detected in all regions of the country, but it was in the Lisbon and Tagus Valley region (n=753; 79%) that the highest number was recorded. The MPXV detection was more frequent in lesion swabs (n=986; 58.1%), oropharyngeal swabs (n=596; 35.1%), rectal swabs (n=75; 4.4%), and urines (n=14; 0.8%). Lesion and rectal swabs showed lower mean values of Ct (cycle threshold), Ct=24 and Ct=25, respectively, suggestive of a higher viral load, compared to oropharyngeal swabs (Ct=30) and urines (Ct=29). These results demonstrate that the best samples for the detection of MPXV, regarding the current epidemic, are the lesion and rectal swabs.

The Emergency Response and Biopreparedness Unit at the National Institute of Health is the reference laboratory for Orthopoxviruses and has implemented a laboratory algorithm that guarantees an accurate and quick response. This algorithm allowed the diagnosis of the first cases of mpox within hours, playing an essential role in the success of the national response to this outbreak.

In Portugal, the last confirmed case was reported on 17^{th} March 2023. Sero-epidemiological studies will be carried out to monitor the humoral immunity in the Portuguese population in order to understand the epidemiological situation and assess the risk of the resurgence of new cases.

DP 11

Impact of COVID-19 Pandemic on Education: A Learning Case from Indonesian Military Academy

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Objectives: This study aims to evaluate the impact of the Kombipak Yudacov drug regimen and strict health protocols on minimizing COVID-19 infection rates and improving patient recovery at a Military Academy.

Methods: in this single-center, retrospective study, we analyzed data from 559 cadets at the Military Academy over two-year period of the COVID-19 pandemic. Partisipants included COVID-19 positive individuals, 30 of whom exhibited mild symptoms. The effect of the Kombipak Yudacov drug regimen was evaluated along with adherence to rigorous health protocols during isolation.

Results: implementation of the Kombipak Yudacov drug regimen resulted in a significant reduction in hospital stay from an average 14 days to 7 day for asymptomatic individuals and to 10 for those with mild symptoms. This also led to a quicker negative PCR conversion in both asymptomatic and mildly symptomatic patients. Adherence to strict health protocols during isolation, combined with daily monitored exercise routines, ensured overall health maintenance with a 0% mortality rate.

Conclusion: Our findings emphasize the significant role of comprehensive strategies, including effective drug regimens and stringent health protocols, in controlling the COVID-19 outbreak within military

setting. This study provides valuable insights into the effective use of military capacities in a pandemic and proposes a reproducible methodology for capturing health data during such outbreaks. these findings will be instrumental in informing future civil-military cooperation in similar scenarios.

DP 13

Hospital Readiness Of Central Army Hospital Gatot Soebroto, Indonesia In Facing The COVID-19 Pandemic

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On 30 January 2020, the Director General of WHO declared the outbreak of coronavirus disease 2019 (COVID-19) a global public health emergency. COVID-19 was declared a pandemic on 11 March 2020. In Indonesia, President Joko Widodo officially on April 13 2020 declared COVID-19 as a national disaster. The existence of the COVID-19 pandemic has made public health systems globally appear fragile. To deal with a pandemic, WHO developed a Rapid hospital readiness checklist for COVID-19 to help assess overall hospital readiness and to identify a series of priority actions to be taken to be ready for, and respond to, a pandemic. This tool can assess the capacity of health services in dealing with the COVID-19 pandemic. Gatot Soebroto hospital as the highest reference and as the Main Reference for the Presidency needs to be ready to be alert in the fight against COVID-19 because, at the start of the pandemic, many things were still unknown. At that time no one had immunity against COVID-19, the emergence of various hoaxes resulted in obstacles in handling them, there was no proper medicine and no country was ready to face a pandemic, there were many cases of infection in various countries as if countries had no borders, this raises many questions as to whether this is a natural or man-made infection. This paper aims to capture the management perspective of RSPAD Gatot Subroto regarding hospital strategy in dealing with COVID-19 and lessons learned to strengthen hospital resilience during the recovery period of COVID-19. The method used to fulfill the 12 WHO Checklist Standards implemented in the Gatot Soebroto Hospital is by collaborating with various parties, the supra system. Good cooperation has finally been able to produce results in reducing the death rate and increasing the recovery rate. It is known that the key to controlling COVID-19 is wearing a mask, washing hands, and keeping a distance, tracing, tracking, and treatment as well as vaccination. Various innovations developed as a result of being forced by pandemic conditions.

DP 14

Operational researches for improving the ability to deal with environmental infectious diseases in the Japan Self-Defense Forces (JSDF)

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In recent years, the Japan Self-Defense Forces (JSDF) have been working to improve their defense response capabilities in the Kyushu-Okinawa region. To that end, it is essential to improve risk assessments not only for infectious diseases due to the nature of group activities, but also for those of high concerns during outdoor activities in the region. In that context, we introduce our recent researches.

Okinawa Prefecture is well known as an endemic area of Leptospirosis. While most civilian case reports have been associated with river recreation, a large outbreak occurred among U.S. Marine Corps jungle training in the northern part of the main island of Okinawa in 2014. Leptospirosis may also occur during disaster relief activities after flooding. We have begun an attempt to assess the risk of infection among JSDF personnel in the southwestern region.

There are concerns about tick and mite borne diseases (life-threatening SFTS, scrub typhus, and Japanese spotted fever with similar clinical features to scrub typhus). These diseases have spread more extensively. In cooperation with the U.S. Forces Japan (Zama) and the Hiroshima Prefectural Institute of Public Health, we conducted a cross-sectional tick surveillance at the JSDF training ground in Hiroshima Prefecture, where every year cases have been reported among citizens. Finally, we will introduce a cross-sectional study of ticks in the southern Chiba Prefecture where the 1st Airborne Brigade conducts jungle training every year.

DP 15

Navigating Indonesia's COVID-19 outbreak: Insights into effective management and the looming threat of multidrug resistance

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Indonesia is an archipelagic country with 17,505 islands spread across 8,300,000 square kilometers, separated by seas. On April 13 2020, COVID-19 designated as a national disaster through Presidential Decree No. 12 of 2020. However the health technological capabilities in Indonesia came with limited resources, Indonesia had to survive the pandemic.
As of 2020, Indonesia faced significant challenges in dealing with COVID-19 pandemic. Indonesia ranked third highest for indirect Covid death in the world. The country experienced a high number of infections and fatalities, struggling to control the spread of the virus and the overwhelming burden on its healthcare system. Government implemented various measures such as social restrictions, testing and contact tracing to mitigate the impact of the pandemic. However, the situation was challenging and the healthcare infrastructure was strained. The high mortality rate in Indonesia is influenced by the presence of underlying diseases among COVID-19 positive patients, vulnerable age groups, and inadequate healthcare facilities.The COVID-19 cases in Indonesia until June 2023 represent 0.88% of the global cases, with a mortality rate of 0.02% below the world rate (0.9%).
The Indonesian Armed Forces (TNI) were among the first to receive vaccinations due to their direct role in enforcing COVID-19 health protocols. Fast forward to the year 2023, comparison reveals a significant improvement in Indonesia's response to COVID-19 crisis. With the implementation of comprehensive vaccination campaigns and enhanced healthcare protocols helped by the military and stakeholders, the country has made significant progress in containing the virus. Moreover, public adherence to health protocols, including mask-wearing and physical distancing, have played a role in preventing the virus's spread.
The possibility of a COVID-19 outbreak caused by multidrug resistance (MDR) is a concerning issue that warrants attention as it could pose challenges to global health systems. Several studies have highlighted the possibility of MDR in the context of COVID-19 caused by bacteria and fungi, highlighting the need for vigilant surveillance and appropriate antimicrobial stewardship. Efforts to mitigate the risk of MDR outbreaks including conducting further research, promoting appropriate use of antimicrobial drugs and strengthening infection prevention and control measures are needed to address this potential threat.

DP 16

COVID-19 Lab Network: The Tunesian experience and future plans

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The recent SARS-CoV-2 pandemic highlighted some of the weaknesses and strengths of government and public health responses to infectious disease outbreaks. In Tunisia, to monitor the spread of SARS CoV-2, a multidisciplinary collaborative approach was followed since the beginning of the pandemic. The microbiology lab of Charles Nicolle Hospital was designated the national SARS CoV-2 reference lab. From 27.02.2020, the public lab network was gradually developed and implemented with the support of the Ministry of Health. In 2021, 33 public and 124 private labs were integrated into the network. Also, the Mobile Military Laboratory, the Forensic Science Laboratory, the Customs Medical Center, and the Technical Police Lab joined the lab network, which provided the relevant reinforcement at the national level.

The COVID-19 experience was an opportunity to work in a lab network which will now play a role in the response to future outbreaks and pandemics in Tunisia. In the frame-work of the German Biosecurity program the RKI plans to collaborate with this public laboratory network, and provide training and workshops in the safe and quality assured detection and diagnostics of highly pathogenic agents, according to the plans for and the needs of the public laboratory network in Tunisia.

This collaboration will help strengthen and support the response to pathogens of epidemic potential in Tunisia.

DP 17

Food Defense: Protection of food against intentional contamination - Project VoLT

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In the area of food protection, concepts with preventive measures are indispensable. One of these concepts is the widely spread HACCP concept,

which focuses on unintentional contamination of food. However, food can also be affected by intentional alteration through introduction of toxic substances or manipulation of the manufacturing process. The term food defense encompasses all measures to protect food from these intentional contaminations [1,2]. Although there are no explicit legal obligations in Germany to date, many international food standards such as IFS Food require a specific food defense plan in the food establishment for certification [3].

The project *Prevention against food terrorism in Baden-Württemberg* (VoLT) (2020–2023) at CVUA Stuttgart is dedicated to raising awareness for the topic of Food Defense. For the evaluation and implementation of food defense measures, a checklist was developed as a practical tool, which was applied in the context of voluntary expert discussions with selected food and drinking water companies [4,5]. In addition, networking with topic-related contacts was advanced and an internal database on hazardous substances was updated.

The initiated discussion on the topic should be extended to other food companies and the acquired knowledge should be conserved. Therefore, corresponding topic-related work is to be continued at CVUA Stuttgart.

The project VoLT is supported by the *Ministerium für Ernährung, Ländlichen Raum und Verbraucherschutz Baden-Württemberg.*

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DP 18

Viral decontamination methods as part of the DEFERM project

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The aim of the project is to work along different case scenarios in order to address concrete procedures and processes of first responders with actual political focus based on threats caused by pathogenic microorganisms. The scenarios include accidental, deliberate or natural release. This focus grants us the possibility to draw on German and French expertise on first responder side and harmonize procedures between the first responders. The main focus is on the recovery phase including decontamination measures to restore facilities and the environment. So far, no clear recommendations or standard operational procedures have been developed for such scenarios in Europe.

Here, we are focusing on procedures to decontaminate viruses using hydrogen peroxide, UV-C, chlorin foam or peracetic acid. Five viruses have been selected to be tested: Monkeypox virus (MPXV), influenza A (H5N1), SARS-CoV-2 (SC2), rift valley fever virus (RVF), coxsackievirus (COXV) and two bacteriophages used as surrogates for field test: Phi-6 and MS2.

Specimens (stainless steel, glass and plastic) were contaminated with a viral concentration of 10^6 pfu/ml, dried and exposed to decontamination. Cotton swabs were used to recover treated and untreated viruses. Viral quantity was assessed by plaque forming assay or real-time cell analysis.

DP 19

Moroccan public health emergency prevention, preparedness and response system facing the COVID-19 crisis

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As of January 2020, the Morocco Ministry of Health (MoH), based on its previous experiences in preparing for and responding to Public Health Emergencies (Pandemic flu, Ebola, etc.), has developed a national prevention, prepardness and response plan against COVID-19 (COVID19 PPR plan) in accordance with the provisions of the IHR and the WHO recommendations. This plan included 5 areas 1) Governance and coordination; 2) Monitoring and surveillance; 3) Case Management and Infection Prevention and Control; 4) Strengthening human resources skills and 5) Information & communication.

The implementation of this plan through the development of a procedure manual with continuous adaptation according to the evolution of the pandemic has enabled the Ministry of Health to guarantee equitable access of the resident population in Morocco to prevention measures (including vaccination), testing, and proper medical care.

For several months, the activity of SARS-CoV-2 showed significant decrease in Morocco, in particular intensive care units admissions and deaths, suggest-

ing : 1) a high level of immunity through natural infection, vaccination or both, 2) dominance of low-virulence virus strains, and 3) improved clinical case management. This situation agrees with the statement of the WHO Director on the 15th meeting of the IHR Emergency Committee on 05 May 2023, considering that COVID-19 is now an established and persistent health problem that no longer constitutes an Public Health emergency of International Concern.

The response to the COVID-19 crisis highlighted a pressing needs to strengthen preparedness, prevention, response and resilience to public health emergencies. With this regard, the Morocco MoH has drawn up a global operational plan for the strengthening of its capacities for epidemic alert and response. Through this work, we will share our experience as the principal MoH planning body and a national implementation coordinator of the MoH COVID19 PPR plan, and we discuss our way forward to a stronger and resilient health emergency prevention, preparedness and response system at local, regional and national levels.

EP 06

Yersinia prophages and their impact: Properties of 11 temperate phages isolated from pathogenic *Y. enterocolitica* strains

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Yersinia enterocolitica is a heterogeneous species comprising pathogenic and non-pathogenic strains. Previous data suggest that gene exchange may occur in *Yersinia*. Though, only scarce information exist about temperate phages of *Y. enterocolitica,* even though many prophage sequences are present in this species. We have examined 102 pathogenic *Y. enterocolitica* strains for the presence of inducible prophages. Eleven phages were isolated from ten strains belonging the bio/serotypes B2/O:5,27, B2/O:9 and 1B/O:8. All phages are myoviruses showing lytic activity only at room temperature. WGS of the phage genomes revealed that they belong to three groups, which, however, are not closely related to known phages. Group 1 and 2 are each composed of five phages with genome sizes of 43.8-44.9 kb and 29.5-33.2 kb, respectively. While the attachment sites (*attP*) of group 1 phages has only a length of 7 nt, *attP* of phages belonging to group 2 is much longer (50-57 nt). Group 3 contains only one phage whose genome has a size of 36.5 kb, which is moderately similar to group 2. *AttP* of this phage is 11 nt long. Similar to the *attP* sites, the host range of the phages differed significantly. While group 1 phages exclusively lysed strains of bio/serotype B2/O:5,27, phages of group 2 and 3 were additionally able to lyse B4/O:3, some of them even B2/O:9 and 1B/O:8 strains. Initial experiments indicated that some of the phages are able to transmit chromosomal DNA by generalized transduction.

EP 07

Klebsiella-specific phages from fecal samples of animals and their suitability for therapeutic application

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Klebsiella pneumoniae are important bacteria that frequently arise in clinical settings causing severe nosocomial infection. Due to their potential to efficiently exchange genetic material with other organisms, these bacteria force their adaption to prevailing environmental conditions. In the last decades, the number of multidrug-resistant *K. pneumoniae* in clinical settings had increased significantly, leading to a potential limitation of the therapeutical options in the human medicine in the future. Due to the necessity of alternative strategies for the treatment of *K. pneumoniae* infections in human, the suitability of lytic phages is actually studied worldwide. The occurrence of phages in feces samples of animals from Germany collected between 2018 and 2020 was determined by phage activity tests. The recovered phages were characterized in their phenotypic (host rang, plaque formation, TEM) and genotypic properties (WGS) to determine their potential for the treatment of *Klebsiella* spp. bacteria. The occurrence and characteristics of *Klebsiella*-specific phages in fecal samples was investigated to provide the community potential novel phage-prototypes for biocontrol and therapeutical issues. However, the number of recovered phages from fecal samples of the analysed animals seemed to be low. By investigating more than 200 samples, only a few *K. pneumoniae*-specific phages could by recovered from feces. The recovered phages exhibit rather narrow host ranges that are limited to very specific *K. pneumoniae* isolates. WGS revealed that the phages belong to different families of both temperate and lytic genera. Furthermore, sequence analyses indicated that independent from their lifestyles the recovered phages did not carry antimicrobial resistance and/or virulence determinants. Further information on phenotypic and genotypic properties of the phages will be presented. On the basis of the prevailing results, feces samples of animals did not represent a common source for lytic phages against *K. pneumoniae*.

EP 08

Lytic phages from livestock slaughterhouse waste water treatment plants and their suitability to treat multidrug-resistant *P. aeruginosa* isolates

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Pseudomonads are Gram-negative bacteria of the Pseudomonadaceae for which currently 191 valid species have been reported. The species *P. aeruginosa* has a high impact in clinical settings, as they efficiently exchange DNA (i.e. resistance plasmids) with other bacteria. Antimicrobial resistance of multidrug-resistant isolates is multifactorial and based on chromosomal and extrachromosomal factors. Due to the steadily increasing number of multidrug-resistant *Pseudomonas* isolates in clinical settings, efficient strategies for control of these bacteria are needed. As bacteriophages have been reported as suitable alternatives for the treatment of multidrug-resistant isolates, this study aimed to determine the occurrence of lytic phages from waste water treatment plants of poultry and pig slaughterhouses. The recovered phages were characterized in their phenotypic (host rang, plaque formation, TEM) and genotypic properties (WGS) to determine their potential for the treatment of *Pseudomonas* spp. bacteria. In general, all recovered phages exhibited a highly restricted host range, which is limited to the species *P. aeruginosa*. However, the spectrum and the number of lysed isolates vary substantially, and provide thus a good basis for the development of a phage cocktail with broad activity. Overall, the phages were shown to be stable under a wide range of pH and temperature conditions. On the basis of their virion morphology, the phages were allocated to different groups of *Pseudomonas* phages. WGS and bioinformatics analyses revealed detailed information on their phylogenetic relationship to different virus-genera. Furthermore, sequence analyses indicated that the recovered phages are suitable for therapeutic issues as they did not carry antimicrobial resistance and/or virulence determinants. On the basis of the prevailing results, lytic phages can be used to combat *P. aeruginosa* with high efficacy. However, in order to develop a broad active and highly efficient phage compound that prevents development of phage resistant bacterial isolates, the cocktails need to be carefully composed.

EP 09

Comprehensive characterization of a novel *Staphylococcus aureus* phage and phage-driven trade-offs in clinical staphylococcal isolates

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Introduction: Like antibiotics, phages exert an evolutionary selection pressure on bacteria. Understanding phage-driven trade-offs or trade-ups in escape mutant bacteria not only provides important insights into phage-bacterial coevolution but is also essential for pre-clinical evaluation.

Objective: Characterize a novel *Staphylococcus aureus* phage and analyze phage-driven trade-offs and trade-ups in clinical staphylococcal isolates.

Methods: Phage vB_SaH_Mallokai (ΦMallokai) isolated from wastewater was characterized by genome analysis, stability, and electron microscopy. Anti-biofilm activity was assessed by staining, counting colony-forming units, and by using bioluminescent *S. aureus*. To study phage-driven trade-offs, eleven ΦMallokai-resistant *S. aureus* mutants were evolved from clinical pulmonary and orthopedic isolates and compared to their phage-sensitive ancestors for mutations, changes in growth kinetics, morphology, catalase, coagulase, and clumping factor/protein-A production, antibiotic sensitivity, and biofilm formation in vitro and in vivo. Changes in bacterial virulence were determined by murine intraperitoneal/subcutaneous infection models.

Results: ΦMallokai was classified as Herelleviridae by genetic and microscopic analyses. Stability was comparable to other Herelleviridae phages. ΦMallokai exhibited potent anti-bacterial activity against *S. aureus* grown planktonically and as biofilms *in vitro* and *in vivo*. Analyses of the eleven phage-resistant bacterial mutants revealed no trade-ups but several potential trade-offs including increase in Oxacillin sensitivity >10-fold for 5/11 strains.

Conclusion: The novel ΦMallokai exhibited potent antibacterial activity against several clinical *S. aureus* isolates, did not induce deleterious trade-ups, while it frequently induced a beneficial trade-off in

phage-resistant bacteria. *In vitro* and *in vivo* results support the potential clinical use of ΦMallokai for phage therapy.

EP 10

Preparing phage for therapy in infections with multidrug resistant *Klebsiella pneumonia*: the IMB protocol

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The emergence of multi drug resistant (MDR) *Klebsiella pneumoniae (Kp)* strains are a serious challenge for public health and force protection, as MDR associated treatment failures cause high mortality rates in septicemia caused by contaminated wounds and in nosocomial infections. Hence, bacteriophage (phage) therapy has resurfaced as a promising strategy for battling MDR infections as phages have the innate ability to specifically infect and lyse bacteria.

Kp specific phages use the host's capsule, a major virulence factor of *Kp*, as receptor for adsorption. To date, 80 different *Kp* capsule types (K-serotypes) have been described with predominant capsule types varying between different countries and continents. Therefore, therapeutic phages need to be customized according to the locally prevailing variant(s).

At the Bundeswehr Institute for Microbiology (IMB) the research project on therapeutic phages develops and optimizes a protocol that ensures timely care for critically ill patients that still meets the high safety standards we demand of patient care.

The current state of research makes it clear that there is a need for a large number of therapeutic phages for MRGN in particular. The high capsular diversity requires a phage collection that addresses the known receptors. Especially in critically ill patients, the isolation, characterization and provision of a previously unknown phage takes too long to still have a therapeutic benefit.

The IMB production protocol follows best practice guidelines, according to the requirements phages have to meet in European guidelines and those in Germany, to be supervised by the treating physician and used in compassionate phage treatment. This protocol will be the basis of a production pipeline implemented in Munich at INVITRIS - with an emphasis on phages produced using the acellular method, when possible. Furthermore, we conduct studies to expand the host range of phages, e.g. via phage engineering informed by the Appelmans protocol. The optimized protocol and phages designed for purpose provide phage production capabilities to the Bundeswehr and will serve as a blueprint for phage production at BWK Berlin.

EP 11

Modified Appelmans protocol for *in vitro Klebsiella pneumoniae* phage host range expansion leads to induction of a novel temperate linear plasmid prophage vB_tLPPP-KpLi5

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Multi-antibiotic resistant *Klebsiella pneumoniae* (Kp) are widespread due to misuse of antibiotics in patients and livestock.

Using the Appelmans protocol, three T7-like phages with highly colinear genomes were combined in a cocktail and used to infect three Kp growth hosts with different capsule types and five test hosts with the same capsule types, where the phages were initially unable to cause lysis. After 30 cycles of infection, we obtained 5 phages via plaque assay. Four of them were reisolations of the input phages, while the fifth one was capable of lysing KpLi5, which was not lysed by the input phages. Further analysis of its genome revealed a new temperate linear plasmid prophage, vB_tLPPP_KpLi5.

Homologous recombination between the input phages leading to extended hostrange was not observed. The prophage was induced in the presence of all input phages. This has implications for best practice in phage therapy, where linear use of phages instead of using cocktails may prevent stress inductions of less benign temperate phages.

In future work we will try to achieve host range extension through recombination with an adjusted Appelmans protocol in two further experiments. The result of these experiments will show whether phages with extended host range can be generated at all. The information gleaned that way will be used to deliberately design *Klebsiella* phage with extended host range for therapeutic purposes in our Phage4-1 Health program.

EP 12

Rapid and safe production, time-resolved characterization of therapeutic phages against biohazardous and multidrug-resistant bacteria

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The current progress of bacteriophage therapy is hindered by the absence of reliable and safe techniques for phage production, and insufficient phage characterization. To address these limitations, we have developed a versatile cell-free platform that enables the production and analysis of a wide range of phages without dependence on a specific host organism. By coexpressing appropriate host factors, we achieved cell-free expression of selective phage variants targeting gram-positive bacteria.

Cell-free expression also allowed for high-resolution and time-resolved mass spectrometry of non-structural phage proteins, which allowed us to confirm several hypothetical proteins derived from T7 and CLB-P3 phages, and find a novel gene cluster associated with late phage expression. Cell-free phage expression enables accelerated reverse and forward phage engineering, facilitating the safe and customized production of clinical-grade therapeutic bacteriophages.

EP 13

Optimization of bacteriophage production for therapy using a standardized approach and efficacy testing in the *G. mellonella* model

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The increasing antimicrobial resistance (AMR) of bacteria is a serious public health problem in the WHO European Region and worldwide. In this context, *Klebsiella pneumoniae* strains, as part of the ESKAPEE group, are among the leading pathogens associated with the emergence of multiple resistance and nosocomial infections affecting mainly the urinary tract, the lower respiratory tract, the intra-abdominal tract as well as the bloodstream. Especially in view of the increasing emergence of carbapenem-resistant *K. pneumoniae* mediated by various plasmid-encoded carbapenemases, the development of effective alternatives to conventional treatments of bacterial infections with antibiotics should be considered.

A promising alternative to antibiotic therapy could be found in the use of bacteriophages in context of curing infections with multidrug-resistant bacteria. This project will develop and optimize a pipeline for the delivery of personalized phage stocks with the potential for therapeutic use in patients. By using the recombinant reporter phage rTun1::nLuc, which produces the enzyme nanoluciferase upon host infection, individual steps within the pipeline were investigated with regard to their implementation. This included quantification of the endotoxin content and its reduction, experiments to stabilize the phage in various human-compatible media, and determination of the sensitivity of the luciferase system. Related to the endotoxin topic, rebooting of the phage in a non-replicative host was also applied. Another approach to avoid the occurrence of an endotoxic character of the phage, is the acellular packaging of rTUN1::nLuc in collaboration with INVITRIS. A *K. pneumoniae* strain of capsular type 64 is serving as the bacterial host of rTUN1::nLuc, on which the wild-type phage vB_KpP_TUN1(phage TUN1) was isolated from wastewater samples from the intensive care unit (ICU) at the Military Hospital of Tunis (MHT).

The intended use of phages as a medical product is also to be realized long term in the form of phage-infused wound dressings. For the phage efficacy testing in this matrix preliminary *in vivo* infection experiments were performed with the model organism *Galleria mellonella.*

EP 14

Genetically Engineering Bacteriophages to Enhance Their Use as Potential Alternatives to Antibiotics

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With the antibiotic resistance crisis worsening, bacteriophages are being re-examined as potential alternative therapeutics for bacterial infections. The ability to genetically engineer bacteriophages provides potential scope to enhance their efficacy as antimicrobial therapeutics. In this study, a phage was genetically engineered to produce a colicin (antimicrobial protein) during infection of the host bacterium. This colicin is released alongside new phage virions upon host cell lysis, thus increasing the amount of killing agents produced through phage infection. Using the recently developed virulence index method, we have demonstrated that the production of the colicin by the engineered phage resulted in increased virulence compared to

wild-type. Furthermore, treatment of a co-culture with the engineered phage showed killing of a phage-resistant strain, provided a phage-susceptible host was present. As such, the engineered phage treatment exhibited an expanded killing range beyond that of the wild-type phage. This work represents a first step in increasing the efficacy of bacteriophages through genetic engineering to produce additional antimicrobial payloads. Exploration of such techniques may lead to further discoveries on how phages can be augmented to increase their therapeutic potential.

EP 15

Application of modified receptor binding proteins of bacteriophages for the rapid confirmative identification of highly pathogenic bacteria

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Typically, highly pathogenic ('biothreat') bacteria, such as *Bacillus anthracis, Yersina pestis, Burkholderia (pseudo)mallei* or *Brucella spp.,* are routinely identified by culture and polymerase chain reaction (PCR). While identification by PCR is relatively time-efficient, additional confirmative assays can be time-consuming, especially if the presence of intact bacteria is to be detected. Bacteriophage-sensibility assays are being widely used as low-tech add-ons to PCR. However, such assays require at least overnight cultivation and typically depend on pure cultures of suspect pathogen isolates. Phage receptor binding proteins (RBPs) instead of complete phages can facilitate and accelerate phage-based pathogen detection. RBPs may be, e.g., phage tail-fibers or -spikes and are employed by the virus to recognize specific surface structures on bacterial host cells. Here, we employed various RBPs of several commonly used diagnostic phages as specific identification tools for highly pathogenic bacteria (mostly) of risk group 3. Recombinant RBPs were modified by fusing them genetically or enzymatically with reporter proteins, such as fluorescent protein or horseradish peroxidase. We used these RBP-reporters in easy to set up assays for the rapid and highly specific detection of these notorious pathogens not only in pure cultures but also in clinically relevant matrices.

EP 16

Genetic evidence for the interaction between *Bacillus anthracis* encoded phage receptors and their cognate phage encoded receptor binding proteins

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(Bacterio)phages such as γ and AP50c have been shown to infect strains of *Bacillus anthracis* with high specificity, and this feature has been well-exploited in the development of detection assays. To better understand the potential for emergence of phage resistance, and thus the failure of such assays, it is important to identify the host and phage receptors and any additional bacterial genes necessary for phage attachment and entry. Using genetic approaches, the bacterial receptors of AP50c and γ have been identified as Sap and GamR, respectively. An additional AP50c-like phage, Wip1, also appears to use Sap as receptor. The cognate phage encoded receptor binding proteins (RBPs) of Wip1 (P23) and γ (Gp14) and based on genome sequence similarities to Wip1, the P28 protein of AP50c, have been identified. Each of these RBPs have been shown to bind to the surface of *B. anthracis* cells by fluorescence microscopy using mCherry fusions. Here, we present genetic evidence supporting the interaction between Sap and RBPs of AP50c and Wip1 phages since RBP binding is ablated in mutants devoid of Sap protein or other proteins shown to be associated with phage resistance, such as CsaB, Spo0A, Spo0B, and Spo0F. We additionally find that the prophage γBa03 (γ -like prophage) RBP relies on CsaB activity for binding by an as yet unrecognized mechanism. RBPγBa03 binding to *B. anthracis* cells is also unique in that it is not ablated by heat inactivation of vegetative cells, suggesting that its receptor is functional even after heat treatment. These results extend our understanding of the differing genetic requirements for the entry mechanisms adopted by these *B. anthracis* phages.

EP 17

A multi-step production, purification and utilization of *Y. pestis*-specific phages for efficient phage-antibiotic combined treatment of pneumonic plague disease

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Plague pandemics have killed millions of people during the history of humankind. The disease, caused by *Yersinia pestis* bacteria, is currently treated effectively with antibiotics. With the aim of developing alternative treatment modality in the emergency event of a plague outbreak involving antibiotic resistance *Y. pestis* strains, we studied the effectiveness of combined phage and second-line antibiotic treatment in the mouse model of pneumonic plague.

For this purpose, we developed an upstream and downstream phage purification process. All the purification methods used in this study are suitable for the modern demands and regulations of the pharmaceutical and biotechnological industry. Moreover, the downstream process has the feasibility for up scaling for the need of large-scale production. The efficiency of the purified phages ϕA1122 and PST alone or in combination with ceftriaxone, a second-line antibiotic, was evaluated using the mouse model of pneumonic plague. Phage treatment significantly delayed mortality and limited bacterial proliferation in the lungs. However, the treatment did not prevent bacteremia, suggestion that the phage efficiency may decrease in the circulation. Combining phage and second-line antibiotic treatment, which are individually insufficient, provided protection that led to the survival of all infected animals.

The current work presents the development of multi-step process required for phage production and purification following by their utilization for efficient treatment of pneumonic plague disease in the mice model.

EP 18
DUOFAG® — Clinical Trials of Drug Product Containing Bacteriophages

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Antimicrobial resistance (AMR) remains a formidable challenge in managing surgical wound infections caused by S. aureus and P. aeruginosa. Duofag, an innovative bacteriophage-based therapeutic agent, has emerged as a potential solution to combat these resistant pathogens. One of the primary objectives of the Phase 1 and 2 clinical trials for Duofag is to generate robust and statistically significant clinical data, providing compelling evidence for the efficacy of bacteriophages in treating these infections.

Duofag's mechanism of action relies on its unique ability to utilize bacteriophages, highly specific viruses that specifically target and lyse S. aureus and P. aeruginosa bacteria. By selectively eliminating these pathogens while preserving the balance of the microbiome, Duofag offers a promising avenue to address the escalating rates of AMR in infected surgical wounds.

This article provides an overview of the initiation of Phase 1 and 2 clinical trials for Duofag, with a particular emphasis on its therapeutic potential in patients with surgical wound infections. The clinical trials aim to gather comprehensive clinical data, meticulously processed through robust statistical analyses, to demonstrate the efficacy and safety of Duofag.

The focus on infected surgical wounds underscores the critical need for innovative therapeutic approaches, as conventional antimicrobial agents are increasingly falling short due to AMR. Duofag's clinical evaluation seeks to provide tangible evidence of the therapeutic benefits of bacteriophages, paving the way for their integration into clinical practice.

Additionally, the article delves into the preclinical studies that laid the foundation for Duofag's clinical trials and highlights the regulatory considerations involved in its development.

As researchers, clinicians, and investors unite in the fight against AMR, Duofag emerges as a beacon of hope, offering a potential breakthrough in treating S. aureus and P. aeruginosa infections in surgical wound settings. The success of Phase 1 and 2 clinical trials for Duofag is expected to yield compelling clinical evidence, supporting its efficacy and safety, and potentially revolutionizing the treatment landscape for infected surgical wounds.

HP 09
Hybrid capture-based next generation sequencing of *Borrelia* in the United Kingdom

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A key challenge of generating whole genome sequences of organisms such as *Borrelia* directly from patient samples or tick extracts is the low abundance of target DNA and the high abundance of host (e.g. human or tick) material.

Propogation of bacteria using solid or liquid culture media is often used to amplify the target organism in order to generate enough starting material to successfully generate whole genome sequences however key plasmids are often lost during this process due to the lack of selective pressures the organism would encounter during survival in a vector or infection in a host.

PCR amplification is also commonly used to amplify key regions of the target genome to allow genotyping of the organism and to provide enhanced data such as the presence or absence of antibiotic resistance genes however this method only provides information about these target regions and this method is suceptible to issues such as mutations in primer binding sites.

As an alternative to culture and PCR, hybrid capture-based target enrichment allows the enrichment and amplification of target genomes from mixed samples such as patient biopsies and tick extracts and has previously been used to generate whole genome sequences of *B. burgdorferi s.s.* in the United States (Carpi et.al., 2015). For this project, a panel of biotynilated probes were designed specifically focussing on *B. garinii* and *B. afzelii* which are more prevalent in the UK and a range of cultured isolates and tick extracts were sequenced using this method.

Generating additional whole genome sequences of UK *Borrelia* will allow a detailed comparison of different *Borrelia* genotypes found in different regions of the UK and oversees and allow us to determine the suitability of commercially available diagnostic assays including those using recombinant antigens to detect and characterise infections caused by UK *Borrelia*.

HP 10

Two novel *Bartonella* (sub)species isolated from edible dormice (*Glis glis*): Hints of cultivation stress-induced genomic changes

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Bartonelloses are neglected emerging infectious diseases caused by facultatively intracellular bacteria transmitted between vertebrate hosts by various arthropod vectors. The highest diversity of *Bartonella* species has been identified in rodents. Within this study we focused on the edible dormouse (*Glis glis*), a rodent with unique life-history traits that often enters households and whose possible role in the epidemiology of *Bartonella* infections had been previously unknown.

We identified and cultivated two distinct *Bartonella* sub(species) significantly diverging from previously described species, which were characterized using growth characteristics, biochemical tests, and various molecular techniques including also proteomics. Two novel (sub)species were described: *Bartonella grahamii* subsp. *shimonis* subsp. nov. and *Bartonella gliris* sp. nov.

We sequenced two individual strains per each described (sub)species. During exploratory genomic analyses comparing two genotypes ultimately belonging to the same species, both factually and most importantly even spatiotemporally, we noticed significant structural variation between them exceeding the range of our expectations. Based on a detailed study of one such event, we argue that prophage deletion represents the most parsimonious explanation of the observed phenomena.

Moreover, in one strain of *Bartonella grahamii* subsp. *shimonis* subsp. nov. we identified a deletion related to *Bartonella* Adhesin A, a major pathogenicity factor that modulates bacteria-host interactions. Altogether, our results suggest that even a limited number of passages induced sufficient selective pressure to promote significant changes at the level of the genome.

HP 11

Phylogeographic investigation of *Francisella tularensis holarctica* collected in Bavaria between 2020 - 2023

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Tularaemia is a zoonotic disease caused by the Gram-negative bacterium *Francisella tularensis*. In Central Europe, the subspecies *F. tularensis hol-*

arctica is dominating. In Germany, the pathogen is mainly detected in the brown hare (*Lepus europaeus*). As *F. tularensis* mostly leads to the death of these animals, a nickname of the disease here is *rabbit plague*. Tularemia in humans may occur after contact with infected wildlife, frequently in the context of hunting. Other common transmission routes include bites of ticks or insects carrying the agent.

In 2020, tularemia was diagnosed in an infant. Within the scope of this study, ticks were collected around the geographic area of the presumed infection site, the palace park of Nymphenburg, since 2021. The molecular screening revealed *F. tularensis holarctica* in few of the ticks. To study the epidemiological background of the agent, *F. tularensis holarctica* isolated from hares in Bavaria between 2020 and 2023, were further included in the study. Next generation sequencing was carried out including the 56 samples from the present study. Data were combined with 104 *F. tularensis holarctica* genomes from publicly available databases, mainly covering strains from Germany, Switzerland and Austria. Subsequently, comparative molecular investigation included coregenome single nucleotide polymorphism analysis typing using the tools parSNP and SeqSphere. Further phylgeographic analyses were carried out using RAxML, treetime, forest2net, and custom R Scripts. Moreover, using ResFinder genetic signatures indicative for antimicrobial resistances were investigated.As previously published, the phylogenetic ancestors of *F. tularensis holarctica* evolved into a clade extending over the eastern part of the world. One lineage as well branched in Europe and covered the Alpine region as for today. Several of the subclades of F. tularensis holarctica were identified from the hare and tick hosts in the present study. No resistances against relevant antibiotics for the therapy of tularemia were found on the genomic level. This could be confirmed by micro-broth-dilution assays for the 56 new isolates from Bavaria. However, a phylogenetic assignment to a specific geographic area, here the palace park, was not possible. As a conclusion, *F. tularensis holarctica* can be assigned to the known lineages. However, isolates from natural foci reveal limitations regarding a specific phylogeographic assignment.

HP 12

Dig Deeper - Using AI to chart the Pandemics' Data Ocean

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As a *Single Point of Contact,* the Bundeswehr Institute of Microbiology (IMB) has sequenced and analyzed all virus variants of remotely deployed, SARS-CoV-2-positive German soldiers. Doing so, crucial information for the scientific evaluation of mutations and the emergence of novel variants could be collected and implemented to guide strategic and military medical decision making.

These tasks depend on the precise delineation of individual viral outbreaks as well as the global monitoring and tracing of pandemic development and the evolution of genotypic virus lineages at high-resolution. As the pandemic progressed, the exponentially growing body of available genomic sequence data and metadata posed unprecedented challenges.

Besides the fast, but coarse-grained, Machine Learning-based classification of SARS-CoV-2 genotypic variants using Pangolin und Nextclade, IMB has developed and successfully applied graph-based algorithms that allow the fine-grained, spatiotemporal delineation and study of individual outbreaks in the troops in the greater context of the global virus diversity.

The method displays a broad application spectrum, ranging from the surveillance of public health related organisms and viruses to the forensic analysis of potential outbreaks with bioweapon relevant pathogens. The establishment and implementation of data and metadata exchange policies and protocols across command- and administrative-level boundaries is essential to the further development, broader applicability and success of such big data technologies.

HP 13

Genomic surveillance of *Klebsiella* infections in hospital settings

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The importance of genomic surveillance in hospitals for pathogen tracking and antimicrobial resistance control cannot be overstated. In this preliminary study, we collected surface swab samples from an intensive care unit room occupied by two patients diagnosed with *Klebsiella pneumoniae* infections at the Military Hospital of Tunis, Tunisia. To gain a comprehensive understanding of transmission routes within real-world hospital settings, we employed the cutting-edge Oxford Nanopore Technology (ONT) along with a metagenomic approach. Our aims include evaluating microbial diversity through taxonomic profiling, identifying and characterizing

pathogen strains, detecting antimicrobial resistance genes, and comprehending transmission pathways. The findings of this study could potentially contribute to infection control measures in the future by elucidating the spread of pathogens and pinpointing high-risk zones within hospitals. Furthermore, the detection of antimicrobial resistance genes can play a vital role in informing antimicrobial stewardship programs, enabling healthcare providers to make evidence-based decisions regarding the use of antimicrobial agents. Ultimately, this research underscores the necessity and value of genomic surveillance in effectively controlling and preventing healthcare-associated infections.

HP 14

Genomic characterization of clinical *E. coli* isolates harboring *mcr*-1 gene

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Actually, no data on the prevalence of plasmid colistin resistance in Tunisia is available among clinical bacteria. This study aimed to investigate the spread of the *mcr* gene and its genomic characteristics among clinical Gram-negative bacteria (GNB) isolated from six Tunisian university hospitals.

A total of 836 GNB strains were inoculated on COLR agar plates with selective screening agar for the isolation of GNB resistant to colistin. For the selected colistin-resistant isolates, *mcr* genes (*mcr*-1 to *mcr*-9) were screened by multiplex PCR. Whole genome sequencing was done for isolates harboring *mcr* gene by using the DNA prep kit and NextSeq500 sequencer (Illumina).

Colistin resistance was detected in 5.02% (42/836) of the isolates. The *mcr*-1 gene was detected in four *E. coli* isolates (0.59%) that belonged to ST359 (n = 1) and ST2973 clones (n = 3). *The mcr-1* gene was located in the IncI2 and IncX4 plasmid types in one and three isolates, respectively. The genetic environment surrounding the *mcr*-carrying plasmids indicated an unusual lack of mobile insertion sequences. Several other genes conferring resistance to β-lactamines ($bla_{CTX-M-55}$), aminoglycosides (*aph*(6)-Id, *aph*(3")-Ib), phenicols (*flo*R), sulphonamide (*sul*2) and tetracycline (*tet*A) were located on the plasmids. Three isolates were classified as APEC III pathovars with multiple virulence genes [*col*E9, *csg*A, *ets*C, *fde*C, *fim*H, *gad*, *hly*E, *hly*F, *iss*, *iuc*C, *iut*A, *lpf*A, *nlp*I, *omp*T, *pap*A_F19, *shi*A, *shiA*, *sit*A, *ter*C, *ter*C, *tia*, *tra*J, *tra*T, *yeh*A, *yeh*B, *yeh*C, *yeh*D].

This study reports the first description of the *mcr*-1 gene among clinical *E. coli* isolates in Tunisia and highlights the application of the advanced innovation technology of WGS in AMR monitoring and surveillance.

HP 15

Genomic surveillance of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in Tunisia: three years of GISAID data sharing

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At present GISAID is the largest open-access data sharing portal for SARS CoV-2, hosting genome sequences of more than 15 million SARS-CoV-2 strains. We aimed to investigate the diversity of the Tunisian SARS-CoV-2 genome sequences submitted to the GISAID platform until June 20, 2023. In total 2,326 complete genome sequences of SARS-CoV-2 from Tunisian laboratories were downloaded and analyzed.

Overall, 127 lineages and sub-lineages were identified. The pandemic was marked by four waves of infection. The first was associated with B.1 (n=152; 7.1%) ancestral lineages, B.1.160 (n=145;6.7%) and B.1.177 (n=67; 3.1%). The Alpha, Delta, and Omicron Variants of Concern (VOCs) were responsible for most infections and deaths during the second, third, and fourth waves, respectively. The Beta (6 %) and Gamma (n=5%) VOCs, were sporadic in Tunisia and correlated with travelers cases. The Alpha variant (n=531; 24.6%) was firstly detected in January 2020. The Delta variant (n=597;27.7%) circulating during one year, from April 2021 to March 2022. The Omicron variant (n=399;18.7%) was detected in December 2021 and circulating until now in our country. Forty nine Omicron sub-lineages were identified with predominance of BA.2 (n=86;3.9%), BA.5.2 (n=71;3.3%) and more recently XBB.1.9.2 (n=30; 3.9%).

These data summarize the various SARS-CoV-2 lineages circulating in Tunisia during the COVID-19 pandemic. Although the implementation of a genomic surveillance system according to international recommendations is challenging in Tunisia,

sequencing and submission of new SARS COV-2 strains should be continued to detect the occurrence of new variants.

HP 16

Metagenome-assembled genomes from artisanal milk products of Tunisia

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Artisanal milk products pose a significant risk for human health and are the source of several highly-pathogenic bacteria particularly in Tunisia. Metagenomic assembled genomes (MAGs) are essential to culture-free recover near complete microbial genomes, to resolve entirely novel organisms and to link properly metabolic pathways to phylogeny. In this preliminary study, a total of 284 MAGs were recovered using an assembly-based approach. Only those MAGs with more than the 75 % of completeness and less than the 15 % of contamination were selected for further analysis. Not only bacteria typically found in milk microbiomes such as *Lactococcus lactis*, *Lactobacillus delbrueckii*, *Lentilactobacillus kefiri*, *Macrococcus caseolyticus* and *Kurthia* sp., but also potential human pathogens such as *Aeromonas caviae, Acinetobacter johnsonii*, *Acinetobacter junii*, *Moraxella osloensis*, *Klebsiella pneumoniae*, *Streptococcus infantarius*, *Leuconostoc* spp., *Raoultella* sp. and *Enterococcus* spp were recovered as high-quality MAGs. The microbial communities of the artisanal milk products were complex and diverse, consequently only a fraction of the genomes was resolved by this assembly-based approach. For example, *Brucella* spp. reads were detected in some microbiomes but missed with the MAGs. Moreover, our study focused on microbiome associated resistomes and virulence profiles providing valuable pioneer insights into the metagenomes of artisanal milk products from Tunisia.

HP 17

SARS-CoV-2 RNA surveillance in wastewater — Possibilities and opportunities for molecular surveillance

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Close monitoring of dynamic local or regional disease outbreaks can provide detailed insights that are valuable for disease management and containment. To do this, many regular samples from patients and affected individuals have had to be collected and analyzed so far. However, pathogens such as SARS-CoV-2, whose RNA can be detected in the stool of COVID-19 patients and infected individuals, open up the possibility of tracking the dynamics of an infectious event through wastewater screening in a timely manner and without affecting those affected. While this approach is limited in its resolution, i.e. it is not possible to trace positive findings in wastewater back to an individual, the method benefits from its low application hurdle and also has the potential to detect undiagnosed and/or asymptomatic cases (so-called dark figure) within the monitored cohort. Using RNA samples obtained during continuous screening in a military camp of the German Armed Forces, we performed a molecular investigation up to the reconstruction of SARS-CoV-2 genomes or their genotypes. This study provides a retrospective overview of several waves of COVID-19 infections introduced into the camp and shows how the emergence of new virus variants correlates with dynamic global infection activity. It also presents methods for implementing a system for near real-time molecular health surveillance of larger populations, which can also be used for molecular surveillance of other infection parameters.

HP 18

(Fully) automated pathogen identification in environmental samples through non-targeted field-based long read sequencing

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Genomics have started to expand their influence and applications outside the sphere of academic work and research environment. Key to the expansion of genomics and their influence in everyday life, as witnessed during the COVID-19 pandemic, is focused on the development of inexpensive and less complex sequencing pipelines, an increase in the development of straightforward analytical pipelines and the availability of powerful, inexpensive hardware that can perform those computational pipelines. We are living on the dawn of an era where identifying the genomic constitution of a sample will be possible in real-time and without the need of specialised laboratories or personnel. The days of the Tricorder might yet be long to materialise, but Kromek is taking steps in developing a mobile device that is able to receive different environmental sample

types and identify the biological constituents of it, without human intervention.

Kromek has developed an instrument that samples air at 4800 L/min, transfer the respirable range of particles present in the sample in an aqueous solution and extract, purify, amplify, and sequence the nucleic acids present. Sequencing is performed utilising nanopores and signals are base-called, analysed and viral and bacterial pathogens present are identified *in situ.*

Time from sample to identification for DNA is 2 hours and for RNA about 2 hours and 45 minutes. The pipeline can identify a pathogen at a concentration of 5 ppl in the presence of a complex metagenomic sample. The bioinformatics pipeline is constituted of several tools with a final decision algorithm that considers the certainty in the presence of the identified organism in our sample. The probability of identification for most pathogens is over 90% with false positive ratios of at least $10^{-4}$for individual species. The pipeline examines the genomic diversity within each genus class and determines a threshold that is required to be able to identify a particular species with confidence.

Currently, Kromek is modifying the molecular biology and bioinformatics pipelines to analyse samples from multiple environmental types, namely swabs and powders. We are also streamlining the bioinformatics pipeline to reduce the computational requirements and achieve the same results on a laptop.

Kromek is envisioning a world where timely detection of pathogens can minimise the impact of infectious disease outbreaks and strengthen national defences from malicious bioterrorism attacks.

HP 19

Rapid sequencing methods for identification of high-consequence bacterial pathogens

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Introduction: Highly dangerous bacterial agents, including *Bacillus anthracis*, *Yersinia pestis,* and *Francisella tularensis* are classified as category A bioterror agents according to the Centers for Disease Control and Prevention. Human cases pose threats and necessitate a rapid response. We developed rapid sequencing methods and bioinformatics analysis for Nanopore and Illumina platforms using rapid DNA enrichment for direct sequencing from clinical samples. Our goals were to determine the reliability of the methods and to reduce the turnaround time.

Methods: *B. anthracis* (4), *Y.pestis* (4), and *F.tularensis* (4) strains were selected from our strain collection and cultures were spiked into human blood samples. Two different rapid enrichment methods were tested with some modifications in the protocols, namely the Nebnext Microbiome DNA Enrichment kit (New England Biolabs, USA) and repliG (Qiagen, Germany). For library preparation, ONT Rapid barcoding kit (RBK004) and Illumina Nextera XT were used. Sequencing was performed on ONT MK1C and Illumina ISeq devices. CLC genomics workbench (Qiagen) was used for analysis.

Results: The wetlab procedures took 3-5 hours. The sequencing time varied between 3-17 hours depending on the sequencing technology. The platforms yielded comparable sequence quality and bioinformatics analysis took only one hour and resulted in >99% sequence identity and was enough for assembly and annotation of the major virulence and antibiotic resistance genes.

HP 20

Profiling of the *Coxiella burnetii* resistome using a neural network trained on the amino acid composition and PSSM profiles

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Coxiella burnetii is the aetiological agent of Q fever, a zoonotic disease affecting predominantly small ruminants and humans. Treatment of acute and chronic Q fever is based on a combination of doxycycline and hydroxychloroquine therapy. Resistance problems in therapy are rare, but have been described in chronic Q fever cases. Antimicrobial susceptibility testing of *C. burnetii* is difficult due to the strict intracellular growth of the pathogen. Therefore, information on antimicrobial resistance is very limited. In general, the discovery of all antibiotic resistance genes (ARGs) of a pathogen (resistome) is an important approach to support and improve the therapy of infectious diseases.

The 'best hits' approach at the sequence level is the most common method for resistome profiling. However, this approach has not been successful for *Coxiella* genomes. To this end, we developed a machine learning (ML) approach that is based on an artificial neural network, taking advantage of the availability of ARGs databases to improve analytical power, prediction quality and accuracy. Our implementation employed a feature characterization

method based on the amino acid composition of PSSM profiles to encode protein sequences. The evaluation of our model was performed on novel and known ARG sequences, and we obtained an accuracy of *approx* 0.96, as well as high precision and recall.

We applied the model to predict ARGs from 61 *C. burnetii* genomes that were downloaded from the RefSeq database. We observed that most predicted ARGs in extitC. burnetii belong to the multidrug category, followed by macrolide, lincosamide, streptogramin B (MLS) and beta-lactam antibiotics. The predicted multidrug ARGs were mainly efflux proteins with known transmembrane multidrug activity. The beta-lactam ARGs were known lactamases with pseudo-sequences and the predicted MLSs were mainly proteins with ATP-binding activities. The former may explain the well-known inefficiency of beta-lactam antibiotics in Q fever therapy, whereas the latter is somewhat unexpected, since macrolide antibiotics are used as second-line antibiotics in acute Q fever.

HP 21

Update of the CoxBase-plattform with new, improving features for genomic *Coxiella burnetii* analysis

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The zoonotic pathogen *Coxiella burnetii* affects small ruminants and humans, causing *C. burnetii*-mediated disease (coxiellosis and Q fever) worldwide. Molecular Epidemiology to enhance control and study of Q fever was recently improved with our platform CoxBase (https://coxbase.q-gaps.de) which has been designed to address several aspects of the genomic analysis of *C. burnetii* such as epidemiological surveillance, metadata summarization via visualisation, *in silico* implementation of multiple genotyping systems, genotyping data management, and genome annotation. The platform has recently been updated with a new suite of features including the prediction of antibiotic resistance genes using a machine learning approach as well as recent genotyping data information from a plethora of hosts. We will continue to update the platform with new data sources and hope the platform continue to serve as a useful resource to researchers and also to support the worldwide molecular surveillance of Q fever. In addition this database platform could also serve as a supporting analysis and information tool for other pathogens.

HP 22

Operation of the molecular diagnostic laboratory during the war: Kharkiv experience

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Following the outbreak of hostilities in Ukraine, work in eastern Ukraine was organised in a combined mode to continue the diagnosis of infectious animal diseases. Administrative and organisational work was carried out from Khmelnytskyi oblast (western Ukraine), while operational work was carried out directly at the laboratory in Kharkiv. DNARNA Shield was used to transport the test material to ensure long-term storage of the samples. Arrangements were made with private diagnostic companies that had a vehicle and driver to transport some samples. All major equipment was stored in the basement to avoid damage from a blast wave.

Power outages due to the destruction of the electricity infrastructure posed another problem for the operation. To overcome this, we have 3 points of battery power (1 kW - 10 hours of operation) for nucleic acid isolation, amplification, fume hood, electrophoresis and visualisation of results. The laboratory was able to operate thanks to the reagent stocks available, but we found that there was a shortage of lyophilic reagents (amplification kits), as frequent power cuts could render the reagents unusable. Despite all the difficulties, this organisation allowed the investigation of zoonoses such as leptospirosis 0/74, salmonellosis 0/5 and clostridiosis 5/15 in the region. The investigation of the clostridiosis cases showed that the main cause was contaminated fodder (maize, hay) that had been improperly stored as a result of the fighting.

IP 09

Universally applicable, short-term cell culture-based assays for the detection of infectious virus particles

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Determining the infectivity of samples containing (recombinant) virus particles is crucial for their risk assessment. Therefore, we developed universally applicable, short-term cell culture-based infection assays for detection of Adenovirus type 5 (Ad5), Ad41, Vacciniavirus (VACV), Ad5- and Adeno-associated virus serotype 2 (AAV2)-based viral vectors. In these assays, susceptible cells were infected for 3h with infectious virus. As controls, cells were infected for 0h and 3h with samples containing infectious virus and heat-inactivated virus, respectively. Differences in Cq-values (Cq), obtained from virus-specific qPCR analysis, were calculated between infected cells and the two controls. An infection assay with a Cq above 3,3 was considered positive, corresponding to a factor 10 difference in the amount of detected viral DNA.

In cases of infection of cells with Ad5 and an Ad5-based viral vector, Cq $\geq$ 5 was observed between cells incubated with infectious virus for 3h and the controls. The developed infection assay reliably detected 7,88 x 10^3 IU and 2,21 x 10^4 IU of Ad5 and the Ad5-based viral vector, respectively. Moreover, 2,305 x 10^3 IU Ad41 could successfully be detected with the developed assay. Similarly, the infection assay was successful in the detection of an infectious AAV2-based viral vector (Cq $\geq$ 6) and VACV (Cq $\geq$ 7) on cells.

We have developed universally applicable infection assays based on short- cell culture infection. The assays can be applied for the detection of various viruses and viral vectors, and provides a valuable tool in clinical virology and infectious virus diagnostics.

IP 10

Performance of new nucleoprotein-based ELISAs for serodiagnosis of acute Crimean-Congo hemorrhagic fever virus infections

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Introduction: Crimean-Congo hemorrhagic fever virus (CCHFV) causes a highly contagious disease, that is transmitted by ticks and has high case-fatality rates in humans. It is circulating in many Asian and African countries, but also spreading to and within Europe. To cope better with future outbreaks of Crimean-Congo hemorrhagic fever (CCHF), the WHO has pointed out the need for development and validation of CCHF diagnostics, including serological assays. Here, we analyzed the performance of two new nucleoprotein-based Anti-CCHFV IgM and IgG ELISAs (EUROIMMUN).

Methods: Both ELISAs were compared to the VectoCrimean-CHF-IgM and -IgG ELISAs (Vector-Best) using the CCHFV Mosaic 2 IgM and IgG indirect immunofluorescence assays (IFA, EUROIMMUN) as reference. Assay sensitivity was determined using 49 acute-phase serum samples from symptomatic CCHFV-infected patients. The assessment of specificity was based on sera from 30 negative control patients (symptomatic at-risk group), 30 healthy blood donors and 29 patients with hantavirus or sandfly fever virus infections. All samples originated from Turkey.

Results: Sensitivity of the EUROIMMUN ELISAs (IgM: 98.0%, IgG: 47.1%) exceeded that of the Vector-Best ELISAs (IgM: 95.9%, IgG: 35.3%). Specificity for IgM was slightly higher using the EUROIMMUN ELISA (86.4% vs. 84.7%), while both IgG ELISAs yielded a specificity of 100%. Comparison of the quantitative results revealed a very strong positive correlation between both test systems (IgM: r=0.868, IgG: r=0.913), whereas the qualitative agreement was substantial for IgM (84.1%, ?=0.673) and IgG (94.9%, ?=0.791).

Conclusion: The new EUROIMMUN Anti-CCHFV ELISAs are standardized and easy-to-use tools that reliably support the identification of acute CCHF cases, making them suitable for laboratories involved in on-site outbreak support.

IP 11

7 Weeks in 7 minutes: Development of the QIAstat viral vesicular panel for surveillance of Mpox and other lesion associated viruses

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Background: After the outbreak of MPXV Clade II during early May 2022, rapid detection and discrimination from other viral pathogens became critical. The goal of this study was to evaluate the overall analytical performance of the syndromic QIAstat-Dx® Viral Vesicular (VV) Panel (Research Use Only; RUO) in order to detect and discriminate the two MPXV clades I and II among other viral vesicular pathogens (HSV1, HSV2, HHV6, VZV

and Enterovirus).

Methods: Evaluation of inclusivity and exclusivity was done by bioinformatic procedures against available MPXV genomic sequences. Oligonucleotides included in the QIAstat® assay for MPXV detection were included in the analysis to evaluate any possible mutation affecting performance. In addition, a meta-analysis based on BLAST homology was also run to characterize specificity of the assay. Laboratory testing was performed to confirm specificity and sensitivity levels using serial dilutions of MPXV analytical samples.

Results: A total of 6592 genomic sequences from GISAID and NCBI were selected for analysis. Of them, 90 and 6502 corresponded to MPXV clade I and clade II. Oligonucleotide mapping resulted in 100% specificity for both MPXV clades. Only 13 early genomes of clade II were not detected, according to the partial genic deletion found in US/CA and highlighted by CDC. Laboratory testing confirmed the assay specificity, with no cross-reaction with other pathogens. In terms of sensitivity, QIAstat-Dx® VV Panel was able to detect MPXV viral loads below 500 copies/ml.

Conclusions: The QIAstat-Dx® VV Panel was demonstrated to be an efficient tool for MPXV surveillance. Specificity of MPXV detection against other viral pathogens can be assessed in a short time. This system is able to give Ct values for better tracking of infections, thus making the QIAstat-Dx® Viral Vesicular Panel (RUO) a key monitoring tool for the MPXV outbreaks.

IP 12

Detection of Avian influenza strains by the QIAstat respiratory panel for putative spillover of High pathogenic avian influenza strains

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Avian Influenza cases in mammals after contact to infected birds have significantly increase in 2023. So far transmission into human only took place from infected lifestock or wild birds, but first reports were made, that virus isolates showed mutations facilitating replication in mammals. In case there is a further evolution enabling human to human transmission these pathogens have the potential to create the next pandemic.

To assess whether the QIAStat Dx Respiratory panel is capable of detecting High pathogenic Avian Influenza strains *in silicos* assessments were conducted together with testing of virus isolates including recent H5N1 pan zoonotic isolates.

Tests with inactivated virus isolates were conducted under BSL3 conditions.

Results: The in silico assessment of Sequences associated to H1N1, H1N1 pdm, H2N2,H3N2, H5N7, H9N2, H5N8 and H10N7 have shown theoretical coverage which was confirmed by test with artificial DNA sequences covering the Assay binding site.

Further in vitro testing was conducted with virus isolates representing H5N1, H1N1, H7N7, N9N2 and chimeric H18N11 were detected *in vitro* using the QIAstat panel.

Bat IAVs H17N10, H18N11 were not detected, the isolate H9N2 is phylogenetically less different and was successfully detected with the H1 specific assay of the panel.

In summary the *in silico* as well as *in vitro* assessment confirmed the usability of the QIAstat panel to detect putative cases of virus spillover in humans. A specific assay to detect H5N1 would improve the panel but needs to be developed.

IP 13

Wastewater testing on QIAstat shows valuable data generation using standard panels

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Introduction: Already End of 2020 the presence of COVID-19 genetic material and its correlation of load to incidence was shown making wastewater testing a suitable tool for surveillance. The workflow requires sample collection and processing in a well equipped laboratory.

Here we present a use case for the QIAstat Dx allowing the qualitative testing of Wastewater for different disease associated pathogen panels with low manual interaction in a remote operatable fashion.

Materials and methods: Wastewater was collected in February 2023 over a course of 24h and send under cooled conditions to the laboratory. The sample was parallel processed using a quantification workflow (Power Water Por sample preparation using 400μl of Water and quantification using target assay on the QIAcuity Platform) and direct loading of 600μl in different QIAstat Cartridges.

Results: The QIAstat cartridges tested were all robust and tolerated such a highly inhibitive sample material. Further the panels revealed high pathogenic burden in wastewater and showed differences between wastewater plants.

The usage of AMR heavy panels showed strong prevalence of resistant Pathogens in Wastewater, whereas the Gastrointestinal Pathogen panels could already identify presence of Vibrio Cholerae in one site.

Conclusion: QIAstat allows fast screening for more than 20 pathogens or AMRS in wastewater without then eed of a full equipped lab. Still positive results need monitoring where a digital PCR approach would be an ideal match.

IP 14

Testing the tests: Assessing the impact of mutations in SARS-CoV-2 variants on molecular diagnostics tests

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Diagnostic assays are critical tools used to test, diagnose and treat infectious and other diseases. These assays have been extremely valuable in the current COVID pandemic, not only to provide appropriate health care for infected and symptomatic individuals as needed, but also for implementing public health measures such as testing, tracing, and isolating infected and asymptomatic individuals to prevent further transmission of the virus. Sustained transmission and unhindered proliferation of the pathogen across the population during a continuous ongoing pandemic such as COVID 19 results in many variants with mutations. These mutations may lead to signature erosion, a phenomenon wherein diagnostic tests developed using the genetic sequence of the original pathogen may fail and cause a false negative result in a sample containing a new variant. Indeed, significant false negative results were seen with some assays against specific SARS-CoV-2 variants; e.g., S Gene Target Failure in Alpha and some Omicron subvariants. We have developed a tool called PSET (PCR Signature Erosion Tool) to monitor the performance of diagnostic tests *in silico* using genome sequences of pathogens. More than 15 million SARS-CoV-2 genome sequences have been generated and shared by the scientific community across the world. In this study, we analyzed the signatures of 43 PCR assays distributed across the genome against over 1.6 million SARS-CoV-2 sequences. We present evidence of significant signature erosion emerging in just two assays due to mutations, while adequate sequence identity was preserved in the other 41 assays. Failure of more than one assay against a given variant sequence was rare and mostly occurred in the two assays noted to have signature erosion. Assays tended to be designed in regions with statistically higher mutations rates. In silico analyses over time can provide insights into mutation trends and alert users to the emergence of novel variants that are present in the population at low proportions before they become dominant. Such routine assessment can also potentially highlight false negatives in test samples that may be indicative of mutations having functional consequences in the form of vaccine and therapeutic failures. We will also present Ebola signature erosion data during the 2014 outbreak in Africa in demonstrating the power of real time *in silico* monitoring on diagnostics and the ability to develop new assays rapidly in case of signature erosion.

IP 15

Implementation and clinical evaluation of an Mpox virus laboratory-developed test on a fully automated random-access platform

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While Mpox virus (MPXV) diagnostics were performed in specialized laboratories only, the global emergence of Mpox cases in 2022 revealed the need for a more readily available diagnostic. Automated random-access platforms with fast nucleic acid extraction and PCR have become established in many laboratories, providing faster and more accessible testing.

In this study, we adapted a previously published generic MPXV-PCR as a lab-developed test (LDT) on a NeuMoDx Molecular System and isolated MPXV clones from patient materials. To reduce the handling of infectious material, we evaluated a Viral Lysis Buffer (VLB) for sample pretreatment. We further compared the MPXV-LDT-PCR to conventional real-time PCR, determined its sensitivity and specificity using positive swabs, and assessed its performance using external quality assessment samples.

Pretreatment of samples with 50% VLB reduced MPXV infectivity by approximately 200-fold while maintaining PCR sensitivity. The assay demonstrated a sensitivity and specificity of 100% with no cross-reactivity in the samples tested and per-

formed with a limit of detection of 262 GE/mL. In summary, the assay had a turnaround time of fewer than 2 hours and can easily be transferred to other automated PCR platforms, providing a basis for developing quick assays for upcoming pandemics.

IP 16

Novel disposable LAMP platform for detection of *Yersinia pestis* in the field

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Detection of biothreat agents such as *Y. pestis* in the field by first responders and SIBCRA teams currently relies mainly on lateral flow tests. These antigen-based tests are easy to perform under field conditions in full PPE and require no instrumentation. The drawback is their well-known limited sensitivity. On the other hand, nucleic acid based tests such as PCR or LAMP (Loop-mediated amplification) have a substantially higher sensitivity, but usually require some instrumentation and are therefore not suitable for field use.

Here, in a proof-of-concept study, we adapted the Multitest (SelfDiagnostics GmbH, Leipzig) for detection of *Y. pestis* in the field. The Multitest is a novel disposable LAMP platform, which is as straightforward to use as an antigen lateral flow test. The palm-sized device contains lyophilized reagents and compartmentalized buffers and is powered by two AAA batteries. It performs a LAMP assay in ca 30 min. The readout consists of a lateral flow strip that is sealed inside the cartridge, preventing any cross-contamination.

We field-tested the device in a biothreat scenario at the NATO CBRN Exercise Precise Response in Suffield, Canada. It out-performed the concomitantly used antigen-based lateral flow assay in terms of sensitivity by 2-3 orders of magnitude.

IP 17

Rapid and precise RNA/DNA detection on-site using instrument-free Multitest

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The COVID-19 pandemic has shown how emerging infectious disease could quickly affect global health and economy. New pathogens with pandemic potential are also expected to appear soon. In addition, the widespread use of antibiotics has led to the emergence and diffusion of pathogenic bacteria endowed with multidrug resistance that have now become a dramatic threat to health and safety.

The biggest limitation for sampling and investigation teams is the lack of highly sensitive and specific tests for the point of incidence diagnostics of high consequence pathogens. The hitherto existing lateral flow immunochromatographic tests have been demonstrated to lack sensitivity and are difficult to adapt to novel emerging pathogens. Moreover, point-of-care PCR tests existing in the market require expensive instrumentation, highly trained professionals and are difficult to deploy in remote areas.

SelfDiagnostics Deutschland GmbH has developed a highly innovative rapid molecular test platform Multitest that combines the ease of use of rapid tests with the diagnostic accuracy of PCR laboratory tests. SelfDiagnostics Multitest platform was developed as a universal testing system that allows first responders and teams to assess outbreaks and potential threat situations directly in the field with high precision.

SelfDiagnostics' Multitest platform is a unique and novel technological approach to *in vitro* diagnostics (IVD). The Multitest platform is a solution for fast (up to 30 min) on-site testing for the presence of pathogen nucleic acids in sample analytes. Multitest is a single-use qualitative test that can detect any specific RNA/DNA targets using state-of-the art proprietary technology. The combination of loop-mediated isothermal amplification (LAMP) and immunochromatographic product detection enables the detection of up to 4 different pathogenic/endogenic targets. Multitest produces an accurate and reliable test result in an instrument-free manner. In addition, Multitest applies highly stable molecular compositions allowing cold-chain free transportation, long-term storage at room-temperature and easy handling at up to 30°C.

We work closely with our suppliers to meet individual requirements and demands of our customers through targeted and rapid development. With >10 years of experience of *in vitro* diagnostics device R&D, we aim to bring innovative technologies and products to the market to meet the emerging needs.

IP 18

Infection model - 3D conditioning of lung adenocarcinoma cell lines

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The lack of a suitable and easy to use cell model severely complicates the study of respiratory viral diseases. Many viruses fail to replicate outside their natural host, particularly in standard 2D cell culture. In this study, we aimed for an improved respiratory cell model to facilitate viral cultivation with standard laboratory methods. The lung adenocarcinoma cell lines A549 and Calu3 were cultured as 3D-spheroids to induce a physiological lung phenotype. Since standard virus cultivation is based on 2D cell culture, we re-transferred the 3D-grown cells into a 2D culture. By transcriptomic and proteomic analysis, we investigated the changes in gene and protein expression throughout the three stages (2D-3D-2D) of cultivation. We used previously published respiratory viral entry receptors as reference during the different methodological approaches. Furthermore, we identified new genes and proteins that are highly susceptible to our conditioning approach. In a next step, we will use the generated results to develop and implement a new respiratory infection model to study uncharacterized clinical samples and potential emerging viruses.

IP 19

Proteomic characterization of a new cell culture model to study viral infections

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Investigation of viral infections largely relies on the cultivation and enrichment of viruses in 2D cell culture. For studying viral infections in the respiratory tract, Calu3 and A549 are routinely used lung adenocarcinoma cell lines. Due to their cancerous origin, the viral effects on native human physiology are difficult to investigate. Since it is known that 3D cell culture can reprogram cells to a more physiological phenotype, we followed a 3D cell cultivation approach. We performed in depths proteomic analysis of 2D and 3D cultivated cells (Calu3 and A549) using data independent acquisition in combination with gas phase fractionation on an LC-MS/MS system (Vanquish Neo, Orbitrap Exploris 480, ThermoFisher). By optimizing the gas phase fractions in terms of their m/z range and the resolution of the orbitrap, we were able to identify more than 8,000 proteins in every replicate. This approach is a powerful tool to detect changes on the protein level when transferring cells from 2D to 3D cell culture. Furthermore, a principal component analysis showed that the proteome of cells clearly changes after transferring cells from 2D to 3D. 24 hours after retransferring cells from 3D to 2D, several proteins involved in cell growth and cell cycle regulation were strongly upregulated again. Building onto the key findings of this approach, we aim to develop an adapted lung cell model to facilitate the molecular investigation of viral infections.

IP 20

Optimized fluorescence *in situ* hybridization (FISH) for the detection of *Mycobacterium tuberculosis* under field conditions

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Rapid, sensitive and specific diagnostics of microbiological pathogens under restricted field conditions are essential for the protection of the population in outbreak situations. For confirmed diagnostics of infectious agents, two independent methods are required. Both methods must interrogate different pathogen targets to minimize the risk of false-positive or false-negative results. Common diagnostic methods in the field include highly sensitive polymerase chain reaction (PCR). Since fluorescence *in situ* hybridization (FISH) uses a different target structure (ribosomes) than PCR (DNA), the combination of the two methods is highly suitable for diagnostics. The aim of this study is to optimize FISH for field diagnostics of *Mycobacterium tuberculosis*. For this purpose, we intend to develop a suitable protocol for the inactivation of mycobacteria. For FISH, we plan to design and validate a new probe set for tuberculosis diagnostics in order to ensure specific and reliable field diagnostics. Furthermore, toxic and therefore non-field-suitable components of the hybridization buffer required for FISH need to be replaced by non-toxic components. In addition, we intend to lyophilize the components used for FISH, which then simply have to be dissolved again on site. A successful implementation of these steps may lead to improved diagnostics of *Mycobacterium tuberculosis* and other microbiological pathogens under restricted field conditions.

IP 21

QIAprep&amp: An innovative method com-

bining liquid-based sample preparation and multiplex real-time PCR for fast and simple malaria detection

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In response to the challenges experienced during the COVID-19 pandemic, QIAGEN has developed a rapid, simple, and reliable multiplex RT-PCR solution that enabled accurate detection of the SARS-CoV-2 virus in any molecular laboratory worldwide. In the QIAprep&amp workflow a large amount of the primary sample can be applied to achieve high sensitivity without inhibition by the sample. An internal control can be included to indicate functional amplification reaction and a human sampling control is used to monitor sufficient amount of human material in the sample.

QIAprep&amp includes a fast liquid sample preparation chemistry as well as a multiplex one-step RT-PCR master mix, that can be used to detect both RNA and DNA targets. QIAprep&amp expands the testing capacity, reduces consumable use, and minimizes the time per test. This solution can be used very flexibly, it is compatible with common real-time thermal cyclers and various sample types. Moreover, it can be automated on most common liquid handlers.

In this study we present data demonstrating the use of QIAprep&amp for highly sensitive detection of Malaria parasites in blood samples. *Plasmodium falciparum* cultures have been diluted in negative whole blood and were subjected to the QIAprep&amp workflow in comparison to a column-based isolation of DNA followed by PCR detection. Results indicate similar performance of the two workflows while the QIAprep&amp workflow comprises less pipetting steps and is much faster.

IP 22

Construction of a system for testing the filtration efficiency of filtering face masks against virus particles

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Filtering face masks play an important role in protecting public health from airborne diseases. In particular, SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2) pandemic has increased the importance of masks. In Europe, testing of filtering face masks is performed according to EN 149 standard. For this purpose, the permeability of filtering face masks is determined using paraffin oil as test agent. This test method does not allow any statement to be made about the risk of infection from particles penetrating the mask. Therefore, the use of infectious virus particles as test agent can fill this capability gap. Because experiments with SARS-CoV-2 must be performed in a biosafety level 3 laboratory, feline coronavirus (FCoV) was used here as surrogate. In the mask tests, an FCoV suspension was aerosolized using an aerosol generator that produces particles in a size range that mimics those exhaled by an adult human during breathing. These particles then flowed through a filter cassette into which different filtering face mask materials can be clamped. Virus particles that penetrate the mask material are then washed out in an impinger. Subsequently, the viral RNA was quantified by qPCR. In addition, infectious virus particles were quantified using a plaque assay. The system described here allows determination of the filtration efficiency of different filtering face masks using infectious virus particles. In future experiments, the system described here can also be used for other test agents such as SARS-CoV-2.

IP 23

Automated detection of surrogate neutralizing SARS-CoV-2 antibodies using a competitive chemiluminescence microarray immunoassay

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The SARS-CoV-2 pandemic has highlighted the importance of rapid diagnostic tools, such as serological assays for the detection of neutralizing antibodies, which can prevent the cell entry of SARS-CoV-2.

Therefore, we developed a competitive chemiluminescence immunoassay to detect surrogate neutralizing SARS-CoV-2 antibodies within 7 minutes on our analysis platform MCR?R. In the process, neutralizing antibodies bind to the viral receptor binding domain, inhibiting the binding to the human angiotensin-converting enzyme 2 receptor. The performance of the competitive binding inhibition test was characterized by a set of 80 samples. The results were well distinguishable between positive and

negative samples and in good accordance with those obtained with an ELISA-based neutralization test as well as a commercial surrogate neutralization assay.

We were able to develop a rapid test for the detection of SARS-CoV-2 surrogate neutralizing antibodies in human blood samples. The microarray immunoassay could further be used to detect individuals with a high total IgG antibody titer, but only a low neutralizing titer, as well as to monitor the levels of surrogate neutralizing antibodies after vaccinations. The effective test performance in SARS-CoV-2 seromonitoring outlines the potential for the assay to be adapted to other diseases in the future.

IP 24

Developing a new ELISA format for detecting humoral immunity by Mpox virus infection

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In 2022 a global outbreak of mpox virus (MPXV) occurred. This double-stranded DNA virus belongs to the genus orthopoxvirus (OPV) in the family Poxviridae, and is endemic in West and Central Africa. The zoonotic disease mpox (MPX) is caused by infection through dermal and mucosal contact with infectious lesions of infected humans or animals. Symptoms are similar to smallpox disease, but are milder and generally self-limiting. Severe complications and fatalities have been described but are very rare.

The most common method for the detection of OPV is the use of pox-specific oligonucleotide primers in real-time PCR. For the specific identification and cladistic separation of MPXV, the inclusion protein of type A (ATI or A27L) is suitable.

Additionally, there are several methods available to detect humoral immunity via OPV-specific antibodies, including Hemagglutination Inhibition Assay, Enzyme-linked Immunosorbent Assay (ELISA) and Plaque Reduction Assay. The ELISA is based on the binding between antigen and antibody and provides a quantitative detection of OPV-specific antibodies. The reaction is trackable by an enzyme-labelled detection antibody that binds specifically to the antibody. A substrate is then added to start a colour reaction and the absorbance is measured by using a photometer.

The aim of this work is to develop an ELISA format that can detect MPXV infection precisely and easily applied in almost every diagnostic laboratory, without further equipment or additional training. The detection of anti-ATI antibodies facilitates precise identification of MPXV infections. Here, we present a new approach to detect antibodies against MPXV-infected cells, using the ELISA format. OPV-infected Vero cells were fixed in a 96 well plate presenting the intact virus antigen ATI. Subsequently, diluted test sera from donors that suffered from MPXV infection were tested. Serum samples from Charité with ethics vote were used.

To ensure fixed samples were not biologically active anymore, and to compare the relative utility in the ELISA format three methods to inactivate virus and fix the cells were used and evaluated: Heat inactivation, β-Propiolactone (BPL) and paraformaldehyde (PFA).

In addition, the sensitivity and specificity of this ELISA was be compared with an in-house ELISA at IBM using viral particles instead of infected cells.

Quantitative results will be presented and discussed.

IP 25

Application of Next Generation Phage Display technology to identify peptides that can distinguish between closely related flavivirus species

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Flaviviruses are a large family of viruses, which cause human and veterinary disease and pose a potential risk of death. The transmission route is typically through the bite of arthropods such as ticks or mosquitoes. Flaviviruses are a major cause of emerging and re-emerging viral infections. One of the major issues faced when carrying out serology diagnostics is the risk of cross reactive antibodies among closely related species.

Next Generation Phage Display is a molecular biology technique which combines phage display with next generation sequencing. A phage library is created by inserting peptide sequences into a phagemid vector. Each phagemid packaged within the phage in the resultant library displays a particular peptide on its external surface. The main advantage of phage display is linking the phenotype (peptide binding properties) with genotype (the peptide gene within the phagemid).

Serum antibodies from individuals infected with Zika and/or Dengue virus were immobilised on a solid support and incubated with a phage library displaying random peptides. Following washing steps to remove non-specific binding, the phage were rescued and propagated in bacteria. This process,

called biopanning, was repeated three times. The phage genomes were sequenced using Ion Torrent sequencing and through analysing the sequences, the antigenic peptide regions can be identified. The most antigenically potent sites can then be used to create a diagnostic assay such an ELISA.

IP 26

Development of an indirect ELISA based on the recombinant 28 kDa protein Omp28 from *Brucella melitensis*

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Brucellosis remains an important zoonotic infectious disease causing economic losses all over the word. In addition to the risk of infection from consuming dairy products from diseased animals, it could also be misused in bioterrorist scenarios or as a biowarfare agent effective against humans and animals. Therefore, there is the need for effective diagnostic tools such as enzyme-linked immunosorbent assay (ELISA) to perform brucellosis surveillance studies. The lipopolysaccharide (LPS) in the cell wall of *Brucella* is considered the strongest and most important antigen for the immune response during infection. To overcome the cross-reactive nature of LPS with other bacteria, the outer membrane protein Omp28 of *B. melitensis* was chosen as the recombinant antigen for the development of an indirect ELISA (iELISA). Omp28 was overexpressed in *E. coli*, purified and tested with serum samples positive either against *B. melitensis* or *B. suis* for its efficacy as an antigen in an iELISA. By this Omp28 proved to be immunoreactive. No cross-reaction was detected with sera positive for antibodies against *Francisella tularensis*. Omp28 is validated as a potential antigen for the serodiagnosis of brucellosis. Preliminary results have shown that Omp28 can be over-expressed in an *E. coli* system and purified to near homogeneity in a single step. This lends itself to large-scale antigen production and avoids the biological risk involved in handling Brucella to produce LPS antigen. The next steps are to test a larger number of brucellosis positive sera to address sensitivity as well as sera known to be positive for further known cross-reactive antibodies like *Yersinia* IgG to address specificity in more detail.

IP 27

Establishing a FISH-suspension protocol for the detection of intracellular bacteria in THP-1 macrophage-like cells

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Burkholderia spp., *Francisella tularensis* and *Yersinia pestis* are known facultative intracellular pathogens that can cause serious health conditions in humans and therefore might be misused as biowarfare agents. To better understand the interaction between the intracellular bacteria and the host cells and to deduce new therapies, *in vitro* infection models have been established and commonly used. However, to date, the intracellular bacterial load is mainly determined by antibiotic protection assays and there is a lack of implementation of modern techniques, such as flow cytometry and fluorescence *in situ* hybridisation for its analysis.

Since highly specific antibodies are not available for all pathogens, a new appproach is a flow cytometry-based fluorescence *in situ* hybridisation, or Flow-FISH, that allows high-throughput analysis of various parameters on a single-cell level. Flow-FISH employs fluorescence-labelled probes targeting the rRNA of microorganisms and allows an analysis of the fluorescence via microscopy and flow cytometry.

This work describes a new established protocol for flow cytometry-based fluorescence *in situ* hybridisation in suspension that allows the detection of intracellular microorganisms in THP-1 macrophage-like cells using double-labelled oligonucleotides probes for an increased fluorescence signal. The method combines the advantages of both techniques and is suitable for specific measurements of intracellular growing bacteria. As one possible application data from intracellular antibiotic sensitivity testing in an *in vitro* infection model is presented.

JP 06

Evaluation of the immune response to SARS-CoV-2 vaccination one year after the booster dose in oncology patients

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Oncologic patients (OPs) have a compromised immune system due to the disease and the therapies. As a result, the immune responses given by vaccinations, including the one against SARS-CoV-2, might be less effective in these individuals than in healthy ones. For this reason, we investigated humoral and T-cell responses in 67 oncology patients (median age: 60 years) with solid tumors undergoing chemotherapy and/or immunotherapy and 33 healthy donors (HDs) (median age: 54 years). Whole blood samples were collected 1 year after the booster dose of anti-SARS-CoV-2 vaccine. Antibodies levels, quantified by Liaison SARS-CoV-2 trimericS IgG assay, were compared to the levels of neutralizing antibodies obtained by the Plaque Reduction Neutralization Test (PRNT); T-cell specific response was assessed on PBMCs using multiparametric flow cytometry. OPs group showed a lower antibodies production (1640 [823-4820] BAU/ml) compared to HDs (2810 [1880-6650] BAU/ml, p=0.026). The same trend was observed by PRNT test: OPs group showed PRNT50 values between 1:80 and 1:5120, while in HDs were comprised between 1:1280 and 1:10240. In OPs, lower percentages of responding T-cells (CD4: p=0.0078; CD8: p=0.0022) and triple positive T-cells (producing simultaneously IFNγ, IL2, TNFα) compared to HDs were observed (CD4: p=0.0067 and CD8: p=0.0003). The obtained results indicated that the booster dose induce seroconversion in OPs despite their antibody levels are lower than those developed by HDs.

JP 07

A simplified and affordable vaccination protocol allowed the control of Coxiellosis in sheep

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Small ruminants are a major source of human infection with *Coxiella burnetii* (Cb). In 2008 and 2012 Q fever in humans was linked to a sheep flock with 650 ewes. Since 2013 annual vaccination of gimmers (replacement yearlings) with Coxevac^{TM} (Ceva Santé Animale) had been introduced. The long-term effect of this minimalistic vaccination schedule was monitored until 2023.

Annually gimmers - except for a sentinel group - were vaccinated twice three weeks apart, no further revaccination was performed. Shedding was monitored by PCR-testing of vaginal/nasal swabs collected hours after parturition. The immune response (PhI/PhII-antibodies, IFN-γ-Recall Assay) was assessed before and after primary vaccination. In 2018, the effect of a revaccination 1, 2, and 3 years after primary vaccination was assessed in groups of each 10 animals.

In 2012/2013 and February 2014 the rate of positive vaginal and nasal swabs was 78/268 and 67/263, respectively. The mean pathogen load in positive samples was $10^{2,6}$and $10^{1,6}$Cb per vaginal and nasal swab, respectively. Thereafter two vaginal (2021/2023) and one nasal swab (2021) tested weak positive. Seroconversion of sentinels to PhII significantly decreased after 2014, and faded out until 2017. Until 2013 vaccination primarily induced a serological PhI$^+$/PhII$^+$-pattern and a strong IFN-response. In contrast, since 2015 vaccination induced a PhI$^-$/PhII$^+$-pattern and a weaker IFN-response. Revaccination in 2018 resulted in a strong increase of both PhI-, PhII-titres and IFN. No difference was observed for groups.

Prophylactical primary vaccination of gimmers allowed a long-term control of infection, although a subliminal infection persisted. Notably, this subliminal infection did not result in a major outbreak. Vaccination initially (2013/2014) boosted a pre-existing immunity resulting in a serological PhI$^+$/PhII$^+$-pattern. Thereafter, as susceptibility increased vaccination induced a weak immune response characterized by a PhI$^-$/PhII$^+$-pattern. However, revaccination induced nevertheless a strong and complete recall immune response. Therefore, it might be considered as an emergency measure.

This study was financially supported by the Free State of Bavaria and the Bavarian Joint Funding Scheme for the Control and Eradication of contagious Livestock Diseases.

JP 08

Generation and *in vitro* characterization of two recombinant MVA candidate vaccines expressing Marburg virus glycoprotein or nucleoprotein

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Marburg Virus (MARV), causative agent of the severe infectious Marburg Virus Disease (MVD), is endemic in several countries in Africa. Although MVD is a rare disease, MARV has a great potential to cause epidemics with high case fatality rates. Since the first documented cases in 1967, regularly occurring outbreaks are reported. Furthermore, within the last years, MVD cases occurred also in non-endemic regions, such as Guinea (2021) and Ghana (2022), which highlights the importance of developing effective vaccines and treatments. Although several vaccines are currently being tested in preclinical and clinical research, no MARV-specific vaccines are licensed for prevention of MVD by now.

Modified Vaccinia virus Ankara (MVA), a well characterized vaccine strain, is a promising viral vector platform for vaccine development against emerging infections, due to its capacity to successfully deliver multiple recombinant antigens and its established clinical safety.

We aimed to generate recombinant MVA candidate vaccines expressing MARV glycoprotein (MVA-MARV-GP) or MARV nucleoprotein (MVA-MARV-NP). The candidate vaccines were generated using our well established MVA vector technology platform and were *in vitro* characterized according to standardized quality control procedures. We could confirm genetic stability, unimpaired protein expression and replicative capacity in chicken embryo fibroblasts (CEF). In the future, immunogenicity of the candidate vaccines will be tested in mice.

JP 09

Generation and *in vitro* characterization of MVA-based vaccines targeting selected influenza A virus hemagglutinin proteins

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Influenza, a respiratory disease mainly caused by influenza A (IAV) and B viruses of the *Orthomyxoviridae*, is still a burden for our societyÂ´s health and economic system. Both, influenza A andÂ B viruses, circulate in mammalian and avian populations, causing seasonal outbreaks with high numbers of cases. IAV is classified into subtypes or serotypes based on their surface antigens hemagglutinin (HA) and neuraminidase (NA). Due to the variability of seasonal IAV annual vaccination is necessary. Although the vaccination rate is high, outbreaks occur regularly, highlighting the need for a more broadly protective vaccine.

Modified Vaccinia virus Ankara (MVA), a well characterized vaccine strain, is a promising viral vector platform for vaccine development against emerging infections, due to its capacity to successfully deliver multiple recombinant antigens and its established clinical safety.

We aimed to generate recombinant MVA candidate vaccines expressing HA antigens from selected IAV subtypes (H2, H5, H7, H9 and H10). HA antigens were inserted into the MVA genome by homologous recombination and recombinant MVA-IAV-HA were purified by serial plaque passaging. *In vitro* characterization was performed in compliance with standardized quality control procedures. We could confirm genetic stability, unimpaired protein expression and replicative capacity in DF-1 cells. In the future, immunogenicity of the MVA-IAV-HA candidate vaccines will be tested in a preclinical mouse model.

JP 10

Establishment of a Nipah virus disease model in hamsters, including a comparison of intranasal and intraperitoneal routes of challenge

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Nipah virus (NiV) is an emerging pathogen that can cause severe respiratory illness and encephalitis in humans with case fatality rates between 40 and 100%. The main reservoir are Pteropid fruit bats, distributed across a large geographical area including Australia, Southeast Asia and Africa. Transmission into humans is widely reported through exposure of infected pigs, ingestion of contaminated food, contact with an infected person and via aerosol. With no approved treatments or vaccines, NiV poses an emerging threat to global health, has epidemic potential and is classified as a CDC/NIAID Category C Bioterrorism agent. *In vivo* modelling will enable an expansion of our interventions against this emerging pathogen. Given variations in the model parameters observed in different inoculation sites, establishment of an optimum challenge route and dose is required. Upon evaluating the hamster model, intraperitoneal in-

oculation demonstrated more rapid dissemination of the virus to wider tissues, when compared with intranasal inoculation. A dose effect was observed between those causing respiratory illness and those resulting in neurological disease sequelae. The data demonstrates the successful establishment of the hamster model of NiV disease for subsequent use to contribute to the evaluation of vaccine and antivirals.

JP 11

Comparative analysis of vaccine-induced neutralizing antibodies against the Alpha, Beta, Delta and Omicron variants of SARS-CoV-2

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The SARS-CoV-2 virus has infected more than 660 million people and caused almost 7 million deaths worldwide as of January 2023. In the course of the pandemic, a number of SARS-CoV-2 vaccines were rapidly developed and several vaccines are currently licensed for use in Europe. However, optimization of vaccination regimes is still ongoing, particularly with regard to the third dose and even further booster vaccinations. At the same time, the near constant emergence of new virus variants poses an ongoing challenge to vaccine efficacy. In this study, we focused on a comparative analysis of the neutralization capacity of vaccine-induced antibodies against four different previous and current variants of concern (i.e. Alpha, Beta, Delta and Omicron) after two and three doses of COVID-19 vaccine. We were able to show that a third vaccination is necessary to maintain the otherwise slowly decreasing immune protection through neutralising antibodies. However, we also observed that the decline in neutralization susceptibility of SARS-CoV-2 variants to vaccine-induced antibodies cannot be significantly improved by three vaccinations. In contrast, a SARS-CoV-2 breakthrough infection between the second and third vaccination results in overall higher levels of neutralizing antibodies with concomitant improved neutralization of virus variants. This is likely due to the circumstance that at the time of this study, all licensed vaccines were still based exclusively on wild type SARS-CoV-2, whereas infections were mainly caused by virus variants. Thus, our data demonstrate the importance of booster vaccinations, but at the same time also emphasize the need for continued adjustment of COVID-19 vaccines to induce neutralizing antibodies protective against current and future virus variants.

JP 12

The Fluctuation of Antibody Titers Against SARS-CoV-2 after Serial Inoculation of BNT162b2 mRNA Vaccine among Health Care Personnel in Hiroshima

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Background: The development of mRNA vaccines against SARS-CoV-2 virus was a turning point in the COVID-19 pandemic. This study aimed to examine anti-SARS-CoV-2 Spike IgG and neutralizing antibody titer levels among Health Care Personnel (HCPs) after serial vaccination with BNT162b2 mRNA (Pfizer, BioNTech) vaccine.

Methods: This longitudinal cohort study recruited a total of 241 HCPs receiving Pfizer vaccine at the Funairi Hospital, Hiroshima, Japan. Out of these, 85 HCPs were randomly selected. Between March 2021 and November 2022, blood samples were collected at 8 time points before and after each dose of vaccination and tested for anti-SARS-CoV-2 Spike IgG and neutralizing antibody titers.

Results: The age of the 85 HCPs ranged from 20 to 65 years; 85.9 % were female. The mean anti-SARS-CoV-2 Spike IgG titer after 2^{nd}, 3^{rd} and 4^{th} dose was 2479, 1773, and 2087 BAU/mL, respectively. A drop in antibody titer was detected at 5 months after 2^{nd} and 3^{rd} dose (186.37 and 669.60 BAU/mL). Mean inhibition rates ranged between 58.9 % and 96.9 % across the time points of sampling. We found significant correlation between neutralizing antibody and anti-SARS-COV-2 Spike IgG titer at all time points except 3 weeks after dose 3.

Conclusions: Our study demonstrates that fluctuation of anti-SARS-CoV-2 Spike IgG titers correlate with mRNA vaccine inoculation. Antibody titers drop at 5 months after each vaccination suggesting the necessity of booster doses in all age groups of population.

JP 13

A review of the real-world safety and effectiveness of Mpox vaccination

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Mpox, an infectious disease caused by the monkeypox virus, results in a smallpox-like illness. Until recently, transmission of this zoonotic disease was limited to a group of countries in central and western Africa. In May 2022, a global outbreak of mpox began, with more than 88,000 cases and more than 140 deaths reported. Transmission has mostly occurred in the context of sexual contact, with men who have sex with men the most affected.

The World Health Organization recommends vaccination of persons at high risk of mpox infection with second- (ACAM2000) or third-generation (MVA-BN or LC16) orthopoxvirus vaccines. In most countries, MVA-BN was the only mpox vaccine used to respond to the mpox outbreak. Here we review published data on the real-world safety profile and the effectiveness of MVA-BN in preventing mpox.

Safety monitoring of MVA-BN in Australia and the US, during a period in which nearly 1 million doses of MVA-BN were administered, demonstrated that the safety profile was in line with that observed during clinical development.

Retrospective cohort-, case-coverage-, and case-control-studies have reported vaccine effectiveness estimates for pre-exposure vaccination ranging from 36-86% for 1 dose and from 66%-89% for 2 doses. In a prospective cohort study, the effectiveness for post-exposure prophylaxis was estimated to be 89%.

In summary, the available real-world data confirm the safety profile of MVA-BN and demonstrate its high effectiveness in preventing mpox.

JP 14

Persistence of vaccinia virus neutralizing antibodies and efficient cross-protective immunity to monkeypox virus

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The threat of monkeypox virus (MPXV) resulting from the outbreak observed in 2022 (MPXV-2022) has prompted a reassessment of the level of immunity in populations. The vaccinia virus (VACV) vaccines were used to prevent smallpox disease and to control its spread, allowing to declare the eradication of the disease in 1980. These vaccines induce cross-reactive and protective immune responses against MPXV and currently represent one of the measure available for MPXV outbreak control. In order to assess the level of immunity in Italian population, we evaluated the persistence of neutralizing antibodies in the military personnel vaccinated more than 40 years.

A study population consisting of 254 healthy adults was designed to assess the persistence of antibodies against vaccinia virus and their cross-neutralizing capability to MPXV at different times after vaccination. The antibody titer considered as positive was the $PRNT_{50}$ titer obtained by the Plaque Reduction Neutralization Assay (PRNT). The challenge viral strain in the PRNT was the vaccinia virus (ACAM2000) and a MPXV strain Clade II isolated from a clinical swab by Defense Institute for Biomedical Sciences.

Our study confirms that first-generation smallpox vaccines induces durable neutralizing response that may persist more than 40 years in vaccinated healthy adults and that these vaccines neutralize MPXV Clade II infection.

JP 15

Relationship between Spike SARS-CoV-2 antibody test and detection of IFN-y production by T cells after vaccination and/or infection with COVID 19: a pilot study

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SARS CoV-2 virus has induced in humans different type of functional T-cell responses which target a wide array of epitopes within all structural and several non structural viral protein. Also licensed COVID-19 vaccines strongly induce the Th-1 skewed Spike (S) reactive T cell responses. We studied the possible correlation between the quantification of SARS-CoV-2 spike antibodies and a commercially aviable IFN-γ release assay produced by T cell (QuantiFERON® (QF) SARS-CoV-2) after COVID-19 vaccination or infection.

Patient and methods: The sample included 19 military personnel (male: 12 female: 7; median age, 46 years) who had been fully vaccinated with different types of vaccine validated. Peripheral venous blood was collected from military personnel after the second vaccination course (at a median of 132 days; 55-310). Serum was used for the quantification of reactive antibodies Anti-Spike (S) protein SARS-CoV-2 (Elecsys® Roche Anti-SARS-CoV-2 S assay) and for the detection of IFN-γ production by SARS-CoV-2 S CD4+ T cells and CD8+ T cells.

Results: Anti-SARS-CoV-2 S antibodies and QF assay they seem to be related studying the optical densities and the amount of neutralizing antibodies. Five samples (19%) are negative for IFN-γ assay (QuantiFERON® SARS-CoV-2), of these

three samples had antibodies Anti-Spike (S) protein SARS-CoV-2 assay <100 BAU. The relationship between the result of the antibody tests (expressed in Binding Antibody Unit (BAU) and the optical densities of the IFN-γ assay was observed. Larger studies will be needed to understand the correlation between cell-mediated immunity and the humoral immune system.

KP 07

Decontamination measures to restore facilities and environment after a natural or deliberate release of pathogenic microorganisms

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DEFERM: New pathogenic microorganisms increase risks in future outbreaks. Effective decontamination thus plays a key role in protecting first-responders and communities. Yet, current methods lack success: They do not prepare for highly transmissible or highly resistant pathogens that cause large case-numbers, are difficult to disinfect and easily cross borders.

The DEFERM research project fills this gap. Targeting viruses and spore-forming bacteria, the Franco-German consortium develops a new rapid detection system, studies and harmonizes no-touch disinfection methods and researches the management of cross-border incidents.

The detection system aims at simplest operability and minimal hands-on time, while providing parallel analyses of multiple samples and PCR based identification of 12 pathogens within 45 minutes to guide decision-making.

The disinfection methods focus on peracetic acid, hydrogen peroxide and Confoam and will be studied for different applications as foam, aerosol and gas. This allows no-touch disinfection also of complex surfaces.

Aiming at cross-border transmission, social scientists compare institutional preparedness in both countries and organizational safety cultures with the help of computational network analysis. Insights will enhance crisis management.

The consortium evaluates these solutions in field tests in France and Germany. Results will guide training and future missions of first-responder organisations.

Funded by ANR and BMBF in 2021–2024.

KP 08

DEFERM: No-touch Disinfection Methods

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Disinfection processes in situations when contamination with highly pathogenic biological agents is suspected, should be effective, safe, fast and user-friendly. Processes that are based on manual wipe disinfection are often found to be labour-intensive and pose a serious risk exposure for the personnel involved. An alternative are so-called no-touch disinfection processes, which are based on semi-automatic procedures in which a disinfectant agent is applied to the contaminated area, e.g. in gaseous form, as a foam or as an aerosol.

A challenge when using such methods is currently the strong variance in effectiveness, which is why they are still used rather rarely. One of the reasons for this is the large number of influencing parameters that result from the specific circumstances of the application area to be treated.

The objectives of the DEFERM project therefore include the development of standard operating procedures for such disinfection processes, which can be made available to the emergency services. Based on the scenarios developed in the project, application-oriented test designs for the disinfection of interior spaces, emergency tents and rescue vehicles are created and three different processes are evaluated:

* Fumigation with hydrogen peroxide * Aerosolization of a peracetic acid based disinfectant * Confoam, a foam-based process developed by the Alternative Energies and Atomic Energy Commission (CEA).

KP 09

Effective disinfection of risk level 4 viruses: Experimental data for improving biosafety measures in high containment laboratories and hospitals

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(Re-)emerging pathogens with high risk implications for public health call for reliable biosafety measures for patient management and laboratories. Disinfection of contaminated surfaces during outbreaks, biological incidences, or likewise routine lab work play a crucial role in preventing further dissemination of the pathogen. However, disinfectants are usually studied on surrogates only, but reliable data for the actual pathogens of interest is often lacking due to the need for a high containment laboratory and the extraordinary effort of working there. With our ongoing study on tenacity and disinfection of risk level 4 viruses such as Ebola, Nipah, and Lassa virus, we aim to provide experimental data for knowledge-based risk assessment.

To investigate the effects of different surfaces, viral particles were applied on several materials found in hospital and laboratory settings (stainless steel, glass, labware plastics, cotton fabric, nitrile gloves, natural rubber, protective suit). At first, stability of the viruses was analysed over time on these materials and under two climatic conditions, representing an air-conditioned environment such as in a hospital or laboratory, and a warmer, humid condition of a European summer day. For the disinfection study, four commercially available and mainly ready-to-use disinfectants representing different groups of active agents are currently being systematically tested (Terralin liquid, Wofasteril SC super, Incidin Foam, Micro-Chem Plus).

LP 08

Anthrax weaponization and public health policy preparations

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Introduction: Human anthrax (ATX) is a rare non-contagious infection, caused by *Bacillus anthracis* spores. Predominance in nature, ease of manufacture and administration, high case fatality, and antimicrobial resistance, make ATX a biothreat. This study's aim was to correlate natural and weaponized ATX events and health policy preparedness in 17 nations.

Methods: Globally reported human anthrax cases from 1917 to 2022 were recovered using Emergent's ATLAS multisource web-program. A time-lined ten language PRISMA search of Pubmed and grey literature for ATX bioterrorism terms, national ATX public health (PH) policies, associated clinical guidance and all post-1917 ATX scientific citations was performed. An ATX management analysis using *Jordan and Adelle*'s policy criteria was then attributed to the 17 nations.

Results: From 1917 to 2022, 128240 notifiable events were reported (peak, 1981: 7935 cases). ATX citations in research literature remained low (x<50) until 2001's letter attacks and a subsequent ten-year peak without natural case correlation (x<551, n=4634; R=-0.24, P=0.31). Global ATX clinical guidance reports remained at low annual volumes (x<4) from 2006. Weaponization of ATX was cited in 377 review articles with growing tendency from 1984. Only Italy lacked human and animal health policies. Enhanced ATX PH surveillance pertained to only Australia, Canada, France, Norway, Spain, and USA. ATX weaponization events timed with increased in-country information but produced policy change in only five nations.

Conclusions: Higher ATX weaponization information volumes were associated with enhanced public health surveillance and deliberate release response plans while natural case tendencies did not correlate with policy formation and literature citations.

LP 09

Analysis of COVID-19 outbreak origin in China in 2019 using differentiation method for unusual epidemiological events

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Objectives: Origin of outbreaks could be natural, accidental, deliberate, and caused by a new or re-emerging bioagent. The aim of this study was the retrospective analysis of whether the COVID-19 outbreak was natural, accidental, deliberate one, or caused by a new or reemerging bioagent.

Methods: Analysis was performed according to the Radosavljevicâ€“Belojevic method for outbreak scoring and differentiation. Data for the application of this method were obtained by literature review in the Medline database for the period from 2000 to 2020.

Results: The analysis of the unusual COVID-19 outbreak shows that the present official assumption of its natural origin is questionable and pointed out to a probability that the pathogen could have also been

accidentally introduced in the human population.

Conclusions: There are no conclusive pieces of evidence about the reservoir of the pathogen or the source of infection. These parameters are essential for the final clarification of the outbreak origin. This study suggests that the COVID-19 outbreak is a consequence of an accidental release of a new COVID-19 virus, probably during the technical accident and/or negligent violation of hygienic norms in the laboratory facility. Further epidemiological, microbiological, and forensic analyses are needed to clarify the COVID-19 outbreak.

LP 10

Infodemics as a Challenge for Military Operations: Lessons Learned from COVID-19 and Strategies for Coping

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The global COVID-19 pandemic has had a drastic impact on human societies and health systems worldwide.

Infodemics can also affect military operations and soldiers, particularly in terms of information warfare and psychological operations. Here are some potential impacts:

1. Disinformation: counterpart actors can purposefully spread misinformation to cause confusion, undermine morale, or hinder military operations. 2. Manipulation of public opinion: infodemics may cause public opinion to turn against military operations or the use of soldiers. 3. Impairing communication: the spread of misinformation can disrupt communication and information sharing within military units. 4. Psychological effects: When faced with a constant barrage of misinformation, soldiers may develop trust issues and feel insecure. This can affect their willingness and motivation to perform their duties effectively.

To meet these challenges, it is important that military organizations take steps to ensure information integrity. This includes fostering critical thinking skills, training soldiers in information assessment and verification, using technology to detect and counter disinformation, and strengthening communication channels and procedures to protect and improve the flow of information. Extensive training and awareness of the impact of infodemics is also critical to making soldiers resilient to disinformation and strengthening their ability to process and assess information.

LP 11

Biological denialism in the Internet in Europe as a possible Kremlin warfare

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Background and methods: Our study employs both qualitative (12 months) and quantitative (5 months) methods to assess digital traditional and social media after 24.02.2022. (1) We assessed qualitatively media releases in Russian about biological weapons and compared them with official documents released by Russia for the Biological Weapon Convention (BWC) meetings. (2) We performed quantitative analysis of the European infosphere between 24.02.–01.08.2022 to measure the effectiveness of external Russian propaganda on causing anxiety and fear in societies the context of biological weapons and food insecurity (3) Additionally we attempted qualitatively material in Polish from 01.02.–31.12.2022 to understand the potential use of misinformation in the context of biological weapons, food insecurity, infectious diseases among Ukrainian refugees and agroterrorism as a form of propaganda.

Results: Due to the lifecycles and content of narration in One Health we can observe adaptive behavior of Russian Intelligence: (1) *Prewar* on refugees diseases; (2) *Fresh* war with the highest interest in all biological concerns with high degree of fear of bioweapon and hunger; (3) *Normalization* phase with the discussion about refugees diseases; (4) *Pre Odessa treaty* phase with intensification of food related issue; (5) *Post Odessa treaty* phase with decrease of all biological narration; (6) *Infection season* phase with returning infections topic and the last (7) farmer protests and food/feed biological quality.

Conclusion: The strategic goals of Kremlin *Biolab* INFOOPS (information operations) were not achieved (i.e. as we see less and less impact on Polish infosphere after failure of BWC consultation). However, fueling polarization and fear in food insecurity, animal breeders' protest and refugees' health may be interpreted in PSYOPS (psychological operations) dimension, so operational goals of Russian intelligence were satisfied as popularity and social consequences of biological denialism raised in 2022 and continue in 2023 (for instance in context of grains).

MP 07

A case of tularemia in a vaccinated person

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Tularemia is a global zoonotic infection caused by Franciella tularensis with varied clinical presentations. Natural foci of tularemia are widespread in Kazakhstan. Here, we report a case of tularemia in a vaccinated lab worker. The 36-year-old woman received a tularemia vaccination in 2018. In October 2021, she experienced enlarged lymph nodes in the left submandibular and cervical regions, gradually increasing in size with discomfort but no pain. Self-administered azithromycin resulted in a slight improvement. In November 2021, an oncologist made a preliminary diagnosis of possible metastasis based on ultrasound findings showing reactive changes and possible metastasis on the left side. Dyskinesia of bile ducts, gallbladder deformation, and bile stasis were detected. Splenomegaly and chronic pyelonephritis were also present. A lymph node biopsy showed lymphoid cell proliferation and dysplasia, raising suspicion of lymphogranulomatosis, but subsequent examinations did not find tumor signs. In January-February 2022, weakness and joint pain persisted. A serological investigation in June 2022 revealed a positive titer for tularemia. The woman sought medical attention for further lymph node enlargement, tenderness, and throat irritation. Physical examination showed erythema, tonsil redness, and a painless cluster of lymph nodes. Abnormalities in lab tests included decreased hemoglobin levels and positive IgG response to Listeria, but negative IgG for Yersinia. Considering symptoms, vaccination history, and test results, the diagnosis is 'Reaction to new infection in the context of vaccination. Tularemia in vaccinated'. Recommendations include further investigation for tularemia, prescribed antibiotics, and follow-up with a local infectious diseases specialist. his case highlights the potential for infection despite vaccination and emphasizes the need for vigilance among lab personnel suspecting tularemia.

MP 08

Myocarditis in long COVID: exploring the impact of physical exercises on cardiovascular complications

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Long COVID has been associated with many complications. One significant concern is the potential link between long COVID and myocarditis. This case report highlights the effect of physical exercise in individuals experiencing prolonged COVID-19 symptoms. In 2021, a 51-year-old female army had a history of COVID-19 and was treated with favipiravir, paracetamol and cough medicine. Patient had received COVID-19 vaccination 3 times. In February 2023, she experienced near syncope four times within the past two weeks. Symptoms occurred when patient sat for more than an hour and accompanied by left-sided chest pain. The results of blood test were within normal limits including thyroid and cardiac enzymes. First Holter test showed 16% premature ventricular contractions (PVC). The first echocardiography on March 7, revealed sinus rhythm with 80 beats per minute heart rate, normal axis, presence of PVC, and an ejection fraction of 58. The treadmill test on March 13, showed more pronounced PVC at rest compared to during activity with 10 METs endurance. The tilt table test on March 20, was negative. An MRI on April 14, showed a left ventricular ejection fraction (LVEF) of 72%, right ventricular ejection fraction (RVEF) of 58%, normal valves, myocardial inflammation, and no fat infiltration. Second Holter test on May 19, showed 11% PVC, leading to the decision to perform an ablation procedure. Patient consumed bisoprolol 5mg/day. On June 19, an electrocardiogram (ECG) was obtained without any premature ventricular contractions (PVC) even after a 2-hour provocation. It was decided not to proceed with the ablation procedure and to discontinue medication. At the end of February 2023, patient had a 6 minutes walking test (6MWT) which only resulted in a distance of 150 meters. Subsequently, a training program consisting of activities like 3 x 6 minutes walking test (6MWT) and activities of daily living (ADL) was conducted at home in early March 2023. The exercise was progressively increased from a speed of 3 km/h to 5 km/h and from 30 minutes to 60 minutes. An endurance test on May 25, 2023, involved running for 12 minutes and covering a distance of 1600 meters. Since June, engaging in physical activity of 10,000 steps per day has been implemented. Conclusion: gradual and continuous physical exercise aids in restoration of cardiac and respiratory function.

MP 09

Microorganisms in Patients with Acute Respiratory Tract Infections in Bamako, Mali

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Introduction: Acute respiratory tract infections (ARIs) are the second most common cause of consultation in Mali. Here, we describe the first spectrum of microorganisms associated with ARIs in all age groups in Mali.

Methods: Patients with ARIs were recruited at CSCOM Yirimadio and Mali Hospital from January to December 2018 in Bamako, Mali. Nose and throat swab specimens were collected from each patient. Common respiratory pathogens, including 19 viruses and 5 bacteria, were screened using real-time multiplex polymerase chain reaction (RT-PCR).

Results and Discussion: A total of 600 patients were involved, of whom 512 (85.3%) were positive for at least one pathogen. *Streptococcus pneumoniae* (356/600, 59.3%) was the most frequently detected pathogen, followed by human enteroviruses, including rhinovirus (162/600, 27%). A study by the GABRIEL network between 2010-2014 showed that *Streptococcus pneumoniae*, RSV, HMPV, IFVA and S. aureus were the main pathogens. Of the samples positive for *Streptococcus pneumoniae*, 182 were typed into 18 serotypes, and 11A/11D was ranked first. This serotype was not included in the 13-valent pneumococcal conjugate vaccine (PCV13) currently in use. Respiratory syncytial virus (RSV) was detected in only 23 samples including 21 RSVA and 2 RSVB.

Conclusion: According to the detection rate of *Streptococcus pneumoniae* and the main prevalent serotypes, the systematic administration of PCV13 in series with PPSV23 would be the right strategy in Mali.

NP 08

Training of specialists is an important factor in ensuring biosafety and biosecurity in Kazakhstan and other countries of Central Asia

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The Kazakh school of microbiologists - specialists in especially dangerous infections is known throughout Central Asia. The National Scientific Center for Especially Dangerous Infections of the Ministry of Health of Kazakhstan (hereinafter referred to as NSCEDI) is the only institute in the Central Asian region that studies especially dangerous infections. The standards for microbiological and laboratory practice developed by NSCEDI have been ensuring biological safety in this region for many decades.

With the opening of the Central Reference Laboratory (hereinafter referred to as CRL) in 2017, personnel training reached a qualitatively new level. International biosafety and biosecurity standards have been introduced into the work of NSCEDI. The supply and exhaust ventilation system with a negative air flow as the basis for the functioning of the laboratory of the 2nd and 3rd levels of biosafety required fundamentally new approaches to training specialists. Practical courses on the formation of microbiological practice, lectures by experienced specialists on especially dangerous infections provide training for specialists at a high level. CRL regularly conducts trainings on biosafety and biosecurity for scientific and laboratory staff, engineering and support staff.

At present, an international training center operates on the basis of the NSCEDI. In 2022, Kazakhstan adopted a law on biosafety. All this contributes to further improving the quality of biosecurity and biosafety in the Central Asian region.

NP 09

Analysis of the annual monitoring for the Crimean-Congo hemorrhagic fever virus in the western and southern regions of Kazakhstan in 2021-2022

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The foci of Crimean-Congo hemorrhagic fever (CCHF) are geographically located in the southern regions of Kazakhstan (Kyzylorda, Turkestan and Zhambyl regions), where the infection of ticks with the CCHF virus, tick species composition and the number of vectors are monitored annually. The objective of our research was to investigate the genetic variants of the CCHF virus in the southern endemic areas, as well as to monitor the spread of the CCHF virus in the western areas of the country (Aktobe, Atyrau and Mangystau regions). In total, 974 (216 pools) ticks from the western regions and

3527 (583 pools) ticks from the southern regions collected during 2021-2022 were investigated. The presence of CCHF virus was detected by real-time quantitative PCR (qPCR) in 1 pool out of 799 pools (0.12%) with *Hyalomma scupense* ticks captured in the CCHF-endemic southern region. In the western regions, CCHF virus was not detected in ticks. The sequencing of incomplete fragments of the S, M and L segments of the CCHF virus in the detected isolate was identified as genotype *Asia - I.* Phylogenetic analysis showed that the isolate obtained in this study is grouped with the isolate from the CCHF patient, which we reported in 2015 (KX129738 Genebank). We concluded that *Asia - I* genotype remains endemic for Kazakhstan and causes disease among humans. Our findings highlight the importance of including CCHF virus sequencing in the annual monitoring system for better understanding the evolution of the virus in the study areas of our country.

NP 10

Strengthening Biosecurity through Mobile Laboratory Capacities: the German Biosecurity Programme in Uzbekistan

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With the start of the 4^{th} phase of the German Biosecurity Programme (2023-2025), Uzbekistan joins the list of partner countries supported by the German Foreign Office to strengthen their national capacities in the field of biosafety and biosecurity. From 2020-2022, the IMB successfully established two rapidly deployable diagnostic laboratories (mobile labs) in Uzbekistan and trained an Uzbek expert team in mobile diagnostics, financed in the framework of the EU CBRN CoE initiative. The current biosecurity project builds on the established and fruitful cooperation between the IMB and two leading Uzbek public health institutes, RSSPMCEMIPD and RIV.

Now, the effort focuses on consolidating and broadening the established capacities. In particular, this will be achieved by: 1) Establishing a training course on mobile diagnostics according to the Train-the-Trainer concept, 2) Strengthening the capacities to identify and mitigate biological risks, 3) Raising awareness in the fields of biosafety and biosecurity, 4) Encouraging scientific cooperation and exchange. With those measures, Uzbekistan will take a significant step to tackle future biosecurity challenges independently and in compliance with international quality and safety standards. Generally, the German Biosecurity Programme provides a great opportunity to strengthen the base of qualified specialists in Uzbekistan and to improve national training curricula as well as global cooperation in biosafety and biosecurity.

NP 11

Experience of the Laboratoire Nationale de Référence des Fièvres Hémorragiques Virales (LNR-FHV) in the response to COVID-19 in Burkina Faso

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Burkina Faso experienced its first confirmed cases of Covid-19 in March, 2020. Supported by our partners, the Centre Muraz' Laboratoire Nationale de Références des Fièvres Hémorragiques Virales (LNR-FHV) played a central role in the response to Covid-19 in Burkina Faso regarding both on-site and military deployment diagnosis. Invited by the Minister of Health and the Minister of Defense, our rapid response team from the G5 Sahel biosecurity network tested soldiers returning from peacekeeping missions for Covid-19.

Therefore, our P3 mobile laboratory was used for the analysis of oro- and nasopharyngeal swabs. Nucleic acids were extracted from the specimens and tested for Covid-19 by RT-PCR. A subset of PCR-positive samples was sequenced to identify the viral variants.

A total of 3,831 samples were analyzed, with the age group of 21 to 30 years and men (78 %) being most strongly represented. There were 398 Covid-19 positive cases, representing 10.39 % of the samples received. During deployment, 167 positive cases were diagnosed in systematic screenings and controls of confirmed cases. Thereof, 41 samples were sequenced and here the variants 19B (56.1 %), 20A (26.8 %) and 20B (17.1 %) were detected.

In summary, the rapid response team of the G5

Sahel bio surveillance network in Burkina Faso supported to maintain the biosecurity in Burkina Faso during this pandemic. Our efforts helped to prevent the spread of the disease among the population, and especially among military personnel.

NP 13

Training of trainers in the Tunisian mobile laboratory

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Tunisia is an important strategic NATO in North Africa. Increasing levels of biological threats from neighboring countries as well as increasing social instability within Tunisia make it fundamental to have a unit which is capable to efficiently disseminate these unclear biological situations from inside and outside of the country. Therefore, rapidly deployable mobile laboratory has been established 2017 in the framework of the German-Tunisian cooperation of the Enable and Enhance Initiative.

In order to sustain the capacities of the rapidly deployable mobile laboratory, a *training of trainers* program was initiated and established in 2019, including didactic and laboratory education of the future trainers in Tunisia. Ultimately, the goal of the *training of trainers* program is the independence from continued German support, but short-term goals include also increasing the number of members who can be deployed at any time and sustainability of laboratory work during prolonged deployments. The effectiveness of this *training of trainers* approach was proven during the COVID-19 pandemic in 2020, when the Tunisian trainer team prepared more than 20 new members of the rapid deployment team for continuous support of the fight against COVID-19.

For the future, the *training of trainers* approach will be broadened in Tunisia, enlarging both the numbers of trainers and laboratory members to sustain an efficient response to unclear biological situations in Tunisia, as well as African partner countries.

NP 14

Project 53 results in Kazakhstan: The concept of biosafety, sustainable development and anti-pandemic effectiveness

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Kazakhstan joined Project 53 in 2017, when European trainers trained Kazakh biosafety trainers. But we already had training and work biosafety experience in Kazakhstan and international organizations, our own biosafety vision and its sustainability. We identified and trained not only laboratory, but also clinical, veterinary and field Biosafety. We also identified customs/border, military, emergency BS&S. We also had a clear vision for sustainable development BS&S system roadmap. In the first cohort of Biosafety trainers there were teachers of medical, veterinary and biological universities, where elective courses on biosafety were created, which later passed into the basic program. Project 53 appeared in time, in Kazakhstan, we conducted trainings in 2018 and 2019, both in laboratory biosafety, and, which is especially important in light of further developments, in clinical biosafety. In COVID-19 pandemic in Kazakhstan, a critical increase in medical cases was during the first months (84 in March 2020; 5880 in June 2020). Almost all the patients were not laboratory but clinical specialists. The training of laboratory specialists for biosafety within the 53 project has borne result. In regions where we managed to train an active cohort of trainers in clinical biosafety, the percentage of COVID-19 cases in medical workers was significantly lower (5,9 - 14,4; $P<0.05$). So, pandemic had confirm our opinion, that the problem of biosafety should not be limited to laboratories only

NP 15

The glovebox in the bacteriology and virology lab of Charles Nicolle hospital of Tunis: from biosecurity project implementation to pandemic preparedness

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The COVID-19 pandemic demonstrated gaps in countries' preparedness against biological threats. The partnership between the German biosecurity program and the microbiology laboratory of Charles Nicolle hospital (HCN) in Tunis aims to improve preventive measures and capacities against future pandemics. In the absence of high containment laboratories (Biosafety level 3 or 4), a glovebox can provide a high level of user protection when processing samples suspected to contain a highly pathogenic agent. Indeed, gloveboxes were used at the start of the SARS-CoV-2 pandemic to prevent laboratory infections, when the pathogenicity of the virus was still unclear and no vaccines were available.

As one of the largest hospitals in Tunis, the HCN stands at the front line of infectious disease outbreaks in Tunisia andwasthe first and only laboratory in Tunisia to perform SARS CoV-2 diagnostics for several months at the start of the pandemic. In case of future outbreaks, it is likely the HCN would also be one of the first laboratories in Tunisia to receive samples suspected to contain other highly pathogenic agents. Therefore the installation of a glovebox and extensive training of Tunisian teams at HCN, provided by the German biosecurity programme, are key elements for effective biosafety and biosecurity at the HCN. Here we describe the role HCN may play in future outbreaks, and report the processes that will be implemented to inactivate highly pathogenic agents using a glovebox.

NP 16

Monitoring emerging zoonotic viruses in Tunisia - A Synergy of the German Biosecurity Programme and the Global Health Protection Programme

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Tunisia is considered to be at high risk for the (re-)emergence of zoonotic viruses due to its role as an important transit country for human travel, livestock trade and wild animal migration. Our activities within the German Biosecurity Programme (BioSP) resulted in higher laboratory safety standards and an expanded diagnostic portfolio, allowing the implementation of studies to monitor the presence and prevalence of highly pathogenic viruses in Tunisia.

We conducted a study on the prevalence of antibodies against the tick-borne Crimean Congo haemorrhagic fever virus (CCHFV) and the mosquito-borne Rift Valley fever virus (RVFV) in livestock in Tunisia. We detected anti-CCHFV antibodies in 8.6% and anti-RVFV antibodies in 2.3% of all tested samples. These results may indicate an important biological threat and lay the foundation for further studies, which we plan to carry out in the current project phase.

Our achievements in the BioSP paved the way for a successful application for funding through the Global Health Protection Programme (GHPP). Our GHPP project SPOT (Harnessing *S*equencing-based technologies for *P*andemic Preparedness using a *O*ne Health-based approach in *T*unisia and neighboring countries) is designed to complement our activities in the BioSP. The combined effort of BioSP and GHPP-SPOT will provide in-depth knowledge of circulating zoonotic viruses and thus facilitate the early identification and neutralization of biological threats posed by emerging agents.

NP 17

Serological and molecular biological investigation of Tick-borne encephalitis in patients with suspected cases of serous meningitis in Kazakhstan

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The family of flaviviruses includes about 50 species and can cause different diseases in humans. All members are closely related and their similarity in the antigenic structure causes difficulties in the diagnosis of the diseases. Tick-borne encephalitis virus (TBEV), a member of the flavivirus family, is the cause of tick-borne encephalitis (TBE). In Kazakhstan (KZ), annually register up to 60 cases of TBE. However, there is a lack of flaviviruses

serological investigation in humans. Our goals were to calculate the prevalence of antibodies against TBEV in patients with suspected cases of serous meningitis.

In our study, we focused on two endemic regions of KZ (East Kazakhstan Oblast and Almaty Oblast (AO)) and a region where TBE cases started being registered only from 2010 (Akmola Oblast). 166 serum and 130 CSF from patients with suspected cases of meningitis were collected. The human samples were tested with ELISA, IIFT, and real-time RT-PCR.

We found seven samples out of 31 belonged to TBEV, and three samples out of 31 were determined as West Nile fever virus (WNFV). For the first time, TBEV was detected in CSF in KZ, also, we confirmed the presence of specific WNFV antibodies in AO.

In this study, we faced nonspecific isolated signals in TBEV IgM that could not be confirmed (5 out of 31). Additionally, three samples shew nonspecific reactions with CMV IgM and EBV IgM. Also, due to our results, we suspected the presence of Omsk hemorrhagic fever on the territory of KZ.

NP 18

Serological evidence of orthohantaviruses in West Kazakhstan region and Almaty city

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The genus of *Orthohantavirus* (family *Hantaviridae*) is geographically widely distributed and presents a significant impact on public health. Many species of small mammals are natural host reservoirs. Rodent-borne orthohantaviruses can cause two distinct forms of disease in humans: hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in Americas. The first human case of HFRS was registered in West Kazakhstan region in the year 2000 and following years HFRS cases were officially registered in the West Kazakhstan region. Meanwhile there is no any data which serotype is causing disease. The aim of this study is investigation of patients with suspected cases of HFRS in West Kazakhstan and in Almaty city.

The study was set up in 2018-2019 in West Kazakhstan region as an endemic for HFRS as well as non-endemic Almaty city. In West Kazakhstan, samples were collected in West Kazakhstan regional infectious disease hospital and in Almaty city samples were collected in infectious disease hospital as well as in nephrology departments of the central hospitals. A suspected case of HFRS was defined having a fever, backache, abdominal pain, thrombocytopenia or/and signs of haemorrhages or/and acute kidney failure. Paired serum samples were collected for serological testing.

In total 139 suspected cases of HFRS patients analyzed as having paired serum samples and completed questionnaire. ELISA based serology testing of serum samples for orthohantavirus showed IgG seropositivity in 23.7% (36/139) and IgM 5% (7/139), in West Kazakhstan region (n=5/57, 8.8%) and in Almaty city (n=2/82, 2.4%) respectively. Positive IgG and IgM samples further tested by Immunoblot and it showed a reactivity for Puumala in nine samples among IgG positive samples. Similarly, among the seven ELISA IgM positive serum samples tested by Immunoblot IgM showed positivity for Puumala in six samples. For the seventh sample, no differentiation by Immunoblot IgM was possible.

Our data showed that Puumala serotype caused HFRS in West Kazakhstan region as well as one positive sample from Almaty city nephrology department. These results highlight that the awareness about orthohantaviruses among treating doctors is important. Moreover, dissemination of suitable case definitions will be important so that healthcare workers will be able to correctly identify potential cases, and as a consequence access to reliable diagnostics needs to be assured.

NP 19

Strengthening of the national capabilities of the partner countries

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Since 2013, in the frame of Global Partnership Against the Spread of Weapons and Materials of Mass Destruction, Georgia is involved in German Biosecurity Programme which helps partner countries to minimize biological threats, such as the intentional misuse of biological pathogens or outbreaks of highly pathogenic diseases. The aim is not only to prevent any misuse of infectious agents that could pose a threat to the world community, but also to strengthen the health capacities of partner countries, thus enhancing their national security. In this context, the German-Georgian joint project:

Network for Strengthening Biosafety & Biosecurity in the Caucasus Region has been established and constitutes a successful collaboration between the Bundeswehr Institute of Microbiology (IMB) and the National Center for Disease Control and Public Health (NCDC). Molecular detection of TBEV, *B. anthracis, Leptospira* and *Clostridium botulinum* was implemented at Lugar Center for Public Health Research so far. Validation of PCR for diagnostics of *Vibrio cholerae* is still in progress. Molecular study to identify and analyze various variants of SARS-CoV-2 over time has been performed using next generation sequencing. Molecular diagnostic laboratory was set up for COVID 19 PCR testing in the Local Sentinel Station (LSS) of the NCDC in Kakheti region.For human resource development and capacity building number of training courses to strengthen personal skills were conducted in Tbilisi, Georgia.

NP 20

Education of Laboratory Personnel in Georgia for Better Health Outcomes

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Education of laboratory personnel in Georgia is crucial for better health outcomes. The COVID-19 pandemic has emphasized the importance of adhering to international biosafety standards. The Richard G. Lugar Center for Public Health Research (CPHR) of the National Center for Disease Control and Public Health (NCDC) in Georgia, in collaboration with the Bundeswehr Institute of Microbiology (IMB) in Germany, has initiated an ongoing training program for laboratory personnel.

The program, conducted in the NCDC regional laboratories of Batumi, Telavi, and Kutaisi from 2021 to 2023, trained 16 personnel. The seminars focused on international biosafety and biosecurity standards, proper handling of biological materials, and development of SOPs. Following the Train-the-Trainer (TOT) model, experienced NCDC staff, engaged in the German-Georgian Biosecurity Project since 2013, delivered the training, sharing their long-term collaboration expertise.

In conclusion, continuous training ensures that laboratory personnel in Georgia stay updated with the latest advancement. The program enhances the Georgian laboratory network's capabilities, contributing to improved public health.

NP 21

Prevalence and molecular characterization of yellow fever virus in Chad

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Yellow fever (YF) is a re-emerging disease present in 13 Latin American and 47 African countries, including Chad. The disease continues to spread in areas where it had previously disappeared, adversely affecting the population, the economy and development. It is caused by the amaril virus, an arbovirus transmitted to humans by mosquitoes belonging to the Aedes and Haemagogus genera. Symptoms include fever, chills, muscle- and headaches, which generally disappear after a few days. However, in the most severe cases, hemorrhagic complications, jaundice and kidney disorders can lead to a high mortality rate.

In Chad, although a yellow fever epidemic was reported in 2013, no studies have been carried out to assess the prevalence of the disease, identify endemic areas or characterize virus variants. It is therefore possible that the virus is circulating in the country undetected, despite the efforts of health authorities. The true prevalence and molecular characteristics of the virus remain unknown. However, Chad has a genomic sequencing platform, an essential technology for identifying new circulating variants and guiding public health measures.

This study aims to generate epidemiological and molecular data on yellow fever in Chad. It will be carried out over a one-year period in the country's 23 provinces, in 2024. Samples will be analyzed at the Laboratoires de Biosûreté et des Epidémies (LaBiEp) using initial screening by ELISA, and confirmation by RT-PCR. Positive samples will be sequenced to identify variants.

This study will provide valuable information on the epidemiology of yellow fever in Chad, and will help the scientific community and health authorities to take appropriate measures aimed at eradicating yellow fever in Chad.

NP 22

Evaluation of the risk of introduction of arboviruses (Zika, Dengue and Chikungunya) in Morocco by an entomological survey carried out at the national level during 2016 and 2017

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Content of the poster:

General objectives of the survey:

* Study the presence and establishment of *Ae. albopictus* and *Ae. aegypti* in the study areas;

* Delimit the distribution area of the vector *Ae. albopictus* at the level of the city of Rabat;

* Follow the temporal dynamics of the vector.

Organization:

The organization of this work required mobilization both at the central level and at the regional level, concerned by this project in 7 regions of the country.

Methodology and choice of study areas:

This choice of the study area was based on the signal of the presence of *Ae. albopictus* in 2016 in Rabat and also areas considered at risk.

Study period:

This survey lasted two years, from 2016 to 2017.

Staff training:

The personnel involved in the survey benefited from training in entomological monitoring techniques for the various mosquitoes, species identification and awareness raising on the problem of mosquito nuisance.

Results: This study confirmed:

* The presence and establishment of a population of mosquito vectors of VZD of the *Aedes albopictus* species in a limited area of the Agdal district in Rabat;

* The entomological surveys carried out in the other study areas were all found to be negative for arbovirus vector species.

Conclusion:

* The results of this study will be presented to all the staff who participated in the study but also to the intersectoral committee for the Integrated Management of Vector Control.

NP 23

Botulism in Morocco, 1999 through 2023: detection of cases and investigations of an epidemic

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Clostridium botulinum is an anaerobic, rod-shaped spore-forming bacterium that produces a potent neurotoxin, leading to a severe form of food poisoning called botulism. Botulism is a rare but life-threatening illness with a high fatality rate. Symptoms include bilateral flaccid paralysis of the arms and legs, as well as difficulty in breathing due to respiratory muscle paralysis. To investigate botulism cases, the National Hygiene Institute routinely tests clinical specimens and suspected food sources for the presence of botulinum toxin (BoNTs).

In 1999, the National Institute of Hygiene detected the first botulism epidemic in Morocco. The food-borne botulism epidemic totaled 78 cases, including 20 patients who died (25%). Food-borne botulism is initially suspected based on the clinical case presentation of the patient. Cases of botulism are confirmed through laboratory identification of the direct presence of BoNTs and/or those clostridia that produce BoNTs in clinical specimens (detecting 18 positives cases in 43 blood samples), or by the presence of BoNTs in suspect food sources consumed by the patient (detecting 1 positive case in 1060 aliment samples).

Eating 'mortadella' has been noticed in 63% of patients and investigations permitted to identify the factory of 'mortadella' as well as the toxin's type B responsible for this poisoning.

More recently, in June 2017 the National Institute of Hygiene laboratories received the case of a family, two girls, 6 and 10 years old, and their mother, 30 years old, who were contaminated with botulinum toxin.

The majority of these investigations have shown that the cause of botulinum poisoning is food poisoning due to the consumption of deli meats contaminated with botulinum toxin.

NP 24

German Biosecurity Programme 2023-25: Activities of the FLI-Riems in Mauritania, Tunisia and Ukraine

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Within the framework of the German Biosecurity

Programme which is funded by the German Federal Foreign Office, the FLI-Riems supports laboratories in Mauritania, Tunisia and Ukraine. A number of highly pathogenic viral agents that are harmful to humans and animals occur naturally in all three countries. The overall idea is that partner laboratories are empowered to detect unusual disease outbreaks at an early stage in order to enable a rapid response. Furthermore, the Biosecurity Programme promotes competencies and the implementation of efficient procedures to strengthen laboratory safety. In this context, we as the federal veterinary research authority, collaborate directly with our veterinary partner institutions in those countries. In Mauritania, for example, the FLI-Riems has already been continuously carrying out capacity building and research activities at the ONARDEP partner institute since 2013. In the current 4th funding phase of the project, MinION sequencing technology will be established on site and we aim to strengthen our collaboration with the National Institute of Public Health (INRSP) and the Bundeswehr Institute of Microbiology (IMB) in the sense of the One Health approach. Likewise, in Tunisia and Ukraine, the emphasis lies on molecular diagnostics of highly pathogenic viral arboviruses and the improvement of biosafety and biosecurity in laboratories. The bacterial counterpart in these countries is covered by the colleagues from the FLI-Jena.

OP 10

Bacteriological examination of the cloacal cavity of free-living reptiles in Kharkiv suburb

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Snakes gut could potentially be to be unique place in terms of its microbiome. European vipers feed primarily on diverse small mammals who are in turn, could be reservoir hosts of numerous potentially dangerous bacteria. Finally, all reptiles in temperate climatic zones are obligatory hibernating, spending almost half year in winter shelters at the temperature slightly above 0°C. The aim of our study was to explore diversity of gut microbiome of reptiles, in a typical habitat, situated in outskirts of large city.

Methods: Cloacal swabs were collected from *Vipera berus nikolskii* (n=45), *Natrix natrix* (n=8) and legless lizard *Anguis colchicus* (n=4). All sampled animals were healthy adults across activity period (March-October) and sampling in one species (*V. b. nikolskii*) had covered all age and sex groups. Sterile swabs were placed into cloaca and transferred to transport medium before cultivation in the laboratory conditions. *Salmonella* isolates were confirmed by PCR using specific primers for the *inv*A gene amplification (L. Cocolin, 2004).

Results: A total 146 isolates were obtained from the cloacal cavity in all 57 sampled animals. The most represented species were *E. coli* (75.4%) and *Proteus spp.* (56.1%). *Salmonella spp.* isolates were found in *V. b. nikolskii* only, being present in 17.7% (8/45) of the sampled population. *Enterobacteriaceae* were also presented by, *Enterobacter* spp. 29.8% (17/57), *Citrobacter* spp. 19.3% (11/57), *Klebsiella* spp. 15.8% (9/57), and *Shigella* spp. 12.3% (7/57), which were found in all examined reptile species. *Pseudomonas aeroginosa* was isolated only from *Natrix natrix*. In some examined vipers *Hafnia spp., Edwardsiella spp., Staphylococcus aureus, Morganella morganii and Streptococcus spp.* were found.

Conclusions: The first survey of cloacal aerobic bacterial flora of reptiles was conducted in Ukraine. The have obtained results indicating on the diversity of bacteria species that could be further investigated regarding zoonotic potential and role of snakes and other reptiles in natural circulation of potential pathogens.

OP 11

Dogs, horses, sheep, and goats as sentinels of Toscana virus circulation in south-eastern France

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Toscana virus (TOSV) is a negative-stranded, enveloped RNA virus of the Bunyaviridae family. The virus is endemic to the Mediterranean countries due to the presence of the sandfly vector of the genus *Phlebotomus*, particularly between the May to October period. Human infection is usually asymptomatic, but it can cause aseptic meningitis and meningoencephalitis. The geographical extension is related to global warming and the expansion of the habitat of sandflies. The aim of our study was to evaluate the TOSV seroprevalence in animals from south-eastern France. In the area exposed to sandflies (departments of Bouches-du-Rhône and Hérault) blood samples were taken from 186 dogs,

142 horses, 18 sheep and 5 goats, in 2022. For comparison, samples were taken from 47 horses from the Marne department and 48 dogs from the Lot. The seroprevalence study was carried out with the virus microneutralization test. Positive threshold was considered with a titer greater than or equal to 1/40. Of the 351 animals exposed, 17 were seropositive (4.8 %) for TOSV antibodies [8/142 horses (5.6%), 2/186 dogs (1%), 6/18 sheep (33.3%) and 1/5 goat (20%)] with the following titles: 1/40 (10), 1/80 (6) and 1/160 (1). None of the 95 animals living in unexposed areas was scored positive. From early April to early June 2022, 6/16 (37%) sheep seroconverted, at the start of the sandfly activity season. Our study indicates that animals are promising sentinels for exposure to TOSV.

OP 12

Serological diagnosis of Japanese encephalitis by detection of specific IgG and IgM antibodies in serum and CSF

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Introduction: Japanese encephalitis virus (JEV) is the most common cause of vaccine-preventable viral encephalitis in Asia and the Western Pacific. Anti-JEV IgM is often determined exclusively during the diagnostic work-up though its presence does not reliably distinguish between acute and past infections. Detection of IgG seroconversion potentially supports the decision, but its presence as a consequence of prior vaccination or non-JEV flavivirus infection must be taken into account. Here, we analyzed the diagnostic value of anti-JEV glycoprotein E (gE) versus anti-JEV non-structural protein 1 (NS1) IgG determination.

Methods: We investigated consecutive serum (n=20, 3–22 days post symptom onset [DPSO]) and single CSF samples (n=9, 5–12 DPSO) from ten Filipino patients, all fulfilling the case definition of Acute Meningitis-Encephalitis Surveillance combined with anti-JEV IgM seropositivity, using the gE-based Anti-JEV ELISA (IgM) and Anti-JEV ELISA (IgG), and the newly developed Anti-JEV NS1 ELISA(IgG) based on recombinant JEV NS1; all assays from EUROIMMUN. Additionally, 155 potentially cross-reactive sera from patients with other flavivirus infections/vaccinations were examined.

Results: 20/20 (100%) serum and 8/9 (88.9%) CSF samples tested positive for anti-JEV IgM. 7/9 (77.8%) and 6/9 (66.7%) sera taken $\leq$ 10 DPSO were positive for anti-JEV gE and anti-JEV NS1 IgG, respectively, whereas 9/9 (100%) sera taken >10 DPSO were positive for both (DPSO unknown for n=2). Seroconversion of IgG combined with IgM positivity was observed in only two patients, indicating acute primary JEV infections. The remaining eight patients had persistently high titers of at least one of either IgG, suggesting an active immune response to JEV. In the control group, the overall positivity rate of anti-JEV gE IgG (63.7%) exceeded that of anti-JEV NS1 IgG (18.6%).

Conclusion: Detection of gE- or NS1-specific IgG in serum can support JEV serodiagnosis, and NS1-based testing is apparently less affected by prior infection with other flaviviruses or by vaccination. This is supported by the lower homology of JEV NS1 to NS1 from other flaviviruses compared to the highly conserved gE. Furthermore, presence of IgM in most CSF samples underlines the diagnostic value of the Anti-JEV ELISA (IgM), which had not been validated for CSF before this study.

OP 13

The spatiotemporal distribution of anthrax in domestic animals and wildlife in Europe with a particular focus on Ukraine

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Anthrax is a serious, notifiable, non-contagious, toxin-mediated zoonotic disease caused by *Bacillus anthracis.* The disease affects mostly grazing livestock and wildlife species and is considered one of the most important biological agents of bioterrorism that could also be potentially misused in biological weapons. In the current study, we analyzed the spatiotemporal distribution of anthrax cases that have been registered between 2005 and 2022 in Europe in animals based on the officially published data by the World Organization of Animal Health (WOAH) and the Food and Agriculture Organization of the United Nations (FAO). In total, there are 267 registered anthrax cases at WOAH in animals in Europe, including 251 cases in domestic animals and 16 in wildlife. The highest numbers of cases were recorded in 2005 and 2016 followed by 2008. The geographical distribution of registered cases revealed that anthrax cases were registered in 25 countries of the European continent in the last 18 years. Albania, Russia, and Italy represented the highest numbers of registered cases, followed by Romania, France, and

Moldova. In Ukraine, anthrax is currently a sporadic infection. 35 cases of anthrax were registered between 1999-2020 in humans. Most frequently human cases were registered in the Kherson region, followed by the Kyiv and Odesa. In non-human sources, there are 28 notifications were registered since 2007 with isolates mainly from soil samples and cattles. The largest number of positive anthrax samples was registered in 2018, and Odesa, which is close to Moldova, had the highest number of cases, followed by the Cherkasy region. The presence of thousands of biothermal pits and burial grounds of fallen cattle nationwide is alarming and favors the re-emergence of new foci. The data extracted and the number of cases identified by WOAH and FAO databases are not matching. Collaboration between FAO-WOAH-WHO is necessary to ensure the consistency and harmonization of data. The genetic analysis of isolates, investigation of susceptibility to antimicrobial compounds, and determination of virulence and pathogenicity factors are required for awareness raising and preparedness.

OP 14

Etiological structure of microorganisms - causative agents of bloodstream infections in Tashkent (2019 - 2021)

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Introduction: An increasing number of hospital mortality cases stem from bloodstream infections (BSIs) with antibiotic-resistant strains of *K. pneumoniae* and *E. faecium*. Antimicrobial resistance poses a threat to the treatment of infectious diseases in patients and is associated with higher rates of morbidity, mortality, prolonged hospital stays, and overall societal damage.

Methods: We investigated 2063 patients at the Center for Antimicrobial Resistance of the Republican Specialized Scientific and Practical Medical Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases from 2019-2021.

Results: In *E. faecium*, resistance to ciprofloxacin (64.2 %) and levofloxacin (91.6 %) was observed. E. faecalis showed resistance to ciprofloxacin and levofloxacin in only 28.5% and 33.3% of strains, respectively. Resistance to ampicillin was more frequently observed in *E. faecium* (78.5 %) compared to *E. faecalis* (57.0 %). Isolated strains of *E. coli* and *K. pneumoniae* exhibited multidrug resistance, particularly to β-lactam agents. *K. pneumoniae* strains showed resistance to ertapenem (50.0 %) and imipenem (61.6 %). We observed resistance up to 100 % to different fluoroquinolones in *E. coli* and *K. pneumoniae*.

Conclusions: The analysis of strain susceptibility to antimicrobial agents shows an overall increase in antibiotic resistance in BSIs. This underlines the increasing therapeutic challenge in fighting BSIs and warrants future research to tackle this problem.

OP 15

Detection of anti-Rift Valley fever virus antibodies in serum samples from patients with suspected arbovirosis infection

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Introduction: Rift Valley fever virus (RVFV) is classified as a category a priority pathogen by the National Institute of Allergy and Infectious Diseases. There are no studies on RVFV seroprevalence in Europe. Here, we screened for the presence of IgG and IgM antibodies against RVFV serum samples from people who were admitted with arbovirus symptoms at the National Institute for Infectious Diseases (INMI) L. Spallanzani, Rome, Italy, August 2022-May 2023.

Methods: Residual serum samples were anonymized, and sub-aliquots were prepared. Each sub-aliquot was tested for anti-RVFV IgG and IgM by indirect immunofluorescence assay (IFA). A serum neutralization assay was used as a confirmation test.

Results: Overall, 80 serum samples were analysed. 8 out of 80 samples (10%) gave a positive result for anti-RVFV IgG, with titres ranging from 1:40 up to 1:1280. Three of 8 (2.6%) samples were confirmed as seropositive by an in-house serum-neutralization assay, with antibody titers ranging from 1:10 to 1:160. All samples were negative for anti-RVFV IgM and RVFV RNA when tested by IFA and real-time RT-PCR, respectively.

Conclusions: Our data indicate an anti-RVFV IgG antibodies prevalence similar to those observed in other countries of the Mediterranean basin. An implementation of surveillance on RVFV arthropod reservoirs and humans could be useful to detect the areas most at risk of outbreak emergence for the benefit of public health.

OP 16

Screening of antibodies against SARS-CoV-2 antigen S1-RBD in samples collected during

COVID-19 pandemic and pre-pandemic from Hail Region, KSA

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Background: The humoral immune response and its mechanism in COVID-19 virus infection in asymptomatic individuals and those with complications remains to be fully understood.

Methods: An ELISA was standardized for the analysis of serum IgG against the surface receptor S1-RBD protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Evaluation for seroprevalence for serum antibodies against S1-RBD antigen was conducted in samples collected during pre-pandemic [n=120; male (n=60) and female (n=60)] and during the COVID-19 pandemic period [n=120; male (n=60) and female (n=60)] from Hail region, Saudi Arabia. Data were correlated with clinical and demographic factors of the subjects.

Results: Significantly higher levels of serum antibodies against S1-RBD were detected in samples collected during pandemic as compared to the age matched samples collected prior to the pandemic. Moreover, inhibition ELISA exhibited significantly ($p < 0.01$) higher inhibition (80â€“88% inhibition) in samples collected during pandemic time as compared to age-matched pre-pandemic samples (32â€–39% inhibition). Antibodies against S1-RBD antigen were detected in approximately 10% of the total pre-pandemic population (males and females). However, subjects > 60 years of age did not show these antibodies.

Conclusions: Increased levels of antibodies against S1-RBD protein were detected in samples collected during pandemic period, although these subjects were not clinically COVID-19 positive. A few samples from pre-pandemic period exhibited antibodies against S1-RBD, suggesting potential prior exposure to other coronaviruses in the region. Reduced levels of anti-SARS-CoV-2 neutralizing antibodies and vaccine effectiveness necessitate continued strategies for improved assay development, country wise screening and surveillance of large populations which would allow management of future potential outbreaks.

OP 17

Prevalence of leptospirosis serogroups among small rodents in Dnipropetrovsk Oblast (2018-2022)

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Introduction: The prevalence of different leptospirosis serogroups is epidemiologically important and can inform control and prevention measures. The purpose of the work was to analyze the dominance of leptospirosis serogroups among small rodents in Dnipropetrovsk Oblast in 2018-2022.

Methods: From 2018-2022, we collected 2267 small rodents and tested serum samples for 14 *Leptospira* serogroups using microagglutination test. Small rodents were selected throughout the year.

Results: In the study of 2267 specimens of small rodents. Overall, 14.6% of the animals tested positive for antibodies to Leptospira. In this study, the most prevalent serogroup was *Leptospira icterohaemorrhagiae* (38.8%), followed by *L. pomona* (15.2%), *L. ballum* (11.2%), *L. javanica* (9.1%), and *L. grippotyphosa* (8.5%). The share of other serogroups was lower: *L. canicola* (6.7%), *L. bataviae* (5.5%), *L. pyrogenes* (3.03%), *L. australis* (1.2%). Other serogroups were not detected.

Conclusions: Leptospirosis remains one of the most widespread particularly dangerous naturally occurring infectious diseases in the Dnipropetrovsk region. Incidence is recorded at a sporadic level. *Leptospira icterohaemorrhagiae* is the dominant serogroup in this study of small rodents in the territory of Dnipropetrovsk Oblast, which is most often the source of the disease in the population. Taking into account the obtained results, it is necessary to continue joint measures with veterinary medicine specialists to prevent the spread and prevention of leptospirosis among the population of the region.

OP 18

The new consultant laboratory for human pathogenic *Vibrio* species

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The family *Vibrionaceae* comprises a large number of different species, twelve of which are known to be pathogenic to humans, including the causative agent of cholera, *Vibrio* (*V.*) *cholerae*. Cholera is caused by cholera toxin-producing *V. cholerae* of serogroups O:1 and O:139. *V. cholerae* of all other serogroups, as well as other human pathogenic species of the genus *Vibrio,* are also called non-cholera vibrios (NCV) which also occur in saline waters of Germany and Europe. In Germany, infections with NCV occur more frequently during the summer months in waters with temperatures above 20 °C. Starting from wounds or injuries of the

skin barrier, invasive transboundary infections may develop. Occasionally, infections with *V. vulnificus* are even fatal in immunocompromised individuals.

Since there was no notification obligation of NCV in Germany until March 2020, a high number of unreported infections in recent years must be assumed. There has also been no comprehensive NCV-specific environmental monitoring to date, so that the actual risk posed by NCV to public health can currently only be inadequately assessed. Here, the newly appointed consultant laboratory for human pathogenic *Vibrio* species will be presented. This includes the diagnostic procedures for typing and characterization of clinical isolates and environmental isolates, as well as the current research activities on different *Vibrio* spp. in Germany.

OP 19

Recurrence of *Coxiella burnetii* ST8 variant in Abruzzo Region after 10 years. Is this an endemic genotype?

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Coxiella burnetii may persist in the environment for years and is highly infectious, with as little as one organism needed to cause clinical infection, making it an attractive organism for use in biological weapon.

Detection of *C. burnetii* and molecular characterization is crucial to identify the link between epidemiological sources and human infection. Here we describe a new target amplification-NGS strategy to obtain allele sequences directly from positive vaginal swab of an infected goat for MST genotyping. PCR purified products were pooled and processed using the Illumina DNA Prep protocol for sequencing through a NextSeq2000 (Illumina). Raw reads were analysed using the GENPAT platform (https://github.com/genpat-it) by trimming, host depletion and assembly. Contigs were then uploaded to the MST database for sequence comparison (https://ifr48.timone.univ-mrs.fr/mst/coxiella_burnetii/blast.html).

The allelic combination obtained was 5 4 2 5 1 5 3 2 4 4. This MST profile is very close to the widespread ST8, but different at Cox56. To date, this variant has been only described in Central Italy for the first time in 2013. This genotype remerged in a new goat farm in the same region after 10 years. ST8 harbours the QpRs plasmid which was only described from patients with chronic disease. This probably reflects the absence of acute clinical manifestations in this area, but particular attention should be payed for long term effects of the circulating strain.

OP 20

Monitoring studies of circulation of tularemia pathogen in Dnipropetrovsk Oblast from 2018 to 2022

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Introduction: Tularemia is an acute naturally occurring zoonotic infectious disease characterized by specific symptoms and a severe course. The aim of the research was to study the circulation of tularemia in the environment from 2018 to 2022 in Dnipropertrovsk Oblast.

Methods: The following methods were used: serological (indirect hemagglutination assay, IHA) for antibody detection and polymerase chain reaction (PCR) for tularemia pathogen DNA detection. The circulation of *Francisella tularensis* in Dnipropetrovsk Oblast from 2018 to 2022 was analyzed (samples for the study were collected in spring-autumn periods).

Results: From 2018–2022, 3087 environmental objects (water, straw, rodents, ticks, pellets) were tested, of which 592 rodents (19.2%), 1712 ticks (55.5%), 570 pellets (18.5%) were studied using IHA and PCR. Of the 265 pools tested by IHA, 66 positive results were obtained (24.9%). Among the positive pools, the percentage of ticks was 34.8%, rodents 45.5%, pellets 19.7%. The highest percentage of positive serological findings in 2018 was 45.5%, among them rodents dominated (53.3%). 279 pools from environmental objects were tested by PCR method, 36 positive results were obtained (12.9%). The share of positive results (DNA) among pools of ticks was 18.3%, among pools of rodents 4.3%.

Conclusions: Research results indicate that ticks and rodents support the constant circulation of the tularemia pathogen in the region. In this connection, there is a probability of cases of tularemia in people.

OP 21

Overview of the environmental *Francisella* sp. isolate W12-1067 — A unique German *Francisella* strain

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Francisella tularensis is an intracellular pathogen causing tularemia in a variety of hosts including humans, rodents and rabbits. The disease tularemia, also known as rabbit fever, is characterized by various clinical symptoms depending on the route of infection and ranging from skin lesions to severe forms of pneumonia. *F. tularensis* ssp. *holarctica* is the most clinically relevant subspecies in European countries including Germany. In 2014, a new *Francisella* isolate, not belonging to ssp. *holarctica*, was found in a water reservoir of a cooling tower in the East of Germany. This isolate W12-1067 (*F*-W12) shows a high sequence identity to a Chinese isolate, which is now referred to as *Allofrancisella guangzhouensis*. The *Francisella* pathogenicity island (FPI), coding for a type VI secretion system (T6SS), has not been found within the genome of *F*-W12, but the strain exhibits a putative alternative T6SS. In Germany, *F*-W12 is classified into the lowest risk group 1 according to the Technical Rule for Biological Agents 466. However, well-known virulence factors, like the lipoprotein MlaA and the metalloprotease FtsH, were experimentally identified for *F*-W12. Moreover, the strain is able to persist in human lung tissue, macrophages and *Acanthamoeba lenticulata*, and can replicate in *Drosophila* S2 cells. In conclusion, *F*-W12 does not seem to be as virulent as *F. tularensis* ssp. *holarctica*, but its putative pathogenicity for humans or animals still needs to be verified.

OP 23

Dr. Johann (Hans) Schneider - Medical Officer and First Describer of Tick-Borne Encephalitis

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Tick-borne encephalitis (TBE; in Germany referred to as early summer meningo-encephalitis) is the most common and important tick-borne viral infection in Europe and Asia. In addition, TBE virus is a pathogen which was studied by Russians as potential biological warfare agent and it is listed on the CDC pathogens list of potential biological warfare agents. The story of its discovery and first description has been predominantly attributed to the Soviet researchers Zilber and Pavlovsky. It is less well known that the first exact clinical description of TBE first epidemiological study were conducted as early as 1931 including the first description of alimentary transmission. This description was made by a specialist for internal medicine in Neunkirchen, Lower Austria, six years before the virus was first detected in the Far East of the Soviet Union (1937). The head of the department of internal medicine, Dr. Hans Schneider, with good reason can be called a pioneer of TBE. TBE as a disease was known in Austria for many years under the name Schneider's disease. For a long time no information on Hans Schneider has been available. Detailed research revealed that Schneider started his medical career as a student of medicine by a military grant. During World War (WW) I he was a member of the medical corps of Austria. Between WW I and WW II he was appointed head of the department of internal medicine in the district hospital of Neunkirchen, Lower Austria. There he made his fundamental discovery of aseptic epidemic meningitis, later identified as TBE. The epidemic appearance of TBE in Lower Austria must be seen as direct result of the social and economic consequences of WW I. During WW II he served as medical officer and later head of the army hospital in Neunkirchen. This study reveals for the first time Dr. Hans Schneider's life, in particular his military career, and his impact of TBE.

OP 24

Health Care-Associated Infections during COVID-19 pandemic at the Intensive Care Unit of CHU P-G, Bamako, Mali

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Hospitalized patients are frequently exposed to Health Care-Associated Infections (HCAIs) that are mainly caused by multidrug-resistant organisms (MDROs). Our aim was to evaluate the frequency of HCAIs as well as the antimicrobial resistance level of bacteria during the COVID-19 pandemic.

Therefore, a prospective study was conducted from September 2020 to June 2021 at the intensive care unit of the Centre Hospitalier Universitaire du Point-G (CHU P-G) in Bamako, Mali. Samples were collected from hospitalized patients and the hospital environment. Microorganisms were subsequently identified and tested for their antimicrobial susceptibility.

Out of 218 patients, more than every fifth developed a nosocomial infection. Main infections in

descending order were bacteremia, urinary tract infections, pneumonia and catheter-related infections. Of 114 pathogenic strains isolated, 59 % were gram-negative bacteria (GNB), 17 % gram-positive cocci (GPC) and 24 % yeasts. In the hospital environment and personnel hands, we isolated GNB in more than 60 % of all cases. The vast majority of the bacteria isolated from the patients showed multidrug resistance, of which almost one third produced expended-spectrum β-lactamase.

The study clearly demonstrates that despite the systematic use of masks and the practice of hand disinfection during the COVID-19 pandemic, HCAIs were frequent at the intensive care unit of CHU P-G with MDROs isolated from patients, environment, and hospital personnel hands.

OP 25

The significance of Ixodoidea ticks in the spread of infectious diseases

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Tick-borne rickettsiosis (TBR), along with tick-borne encephalitis (TBE), coxiellosis and tick-borne borreliosis (TBB), is among the most common tick-borne infections. In contrast to TBE and TBB, the role of TBR and coxiellosis in the infectious pathology of the population is studied rather poorly. In endemic foci of *Coxiella burnetii* and *Rickettsia* spp., cattle infected through ticks on pastures in spring time become a potential pool for human infection. The aim of this work was to determine the spread of TBR and coxiellosis infections in Uzbekistan. A total of 14,967 individual tick specimen were collected from wild animals and livestock. We found 15 tick species from 6 genera of the Ixodoidea superfamily in the studied region: *H. sulcata, H. concinna, R. analatus, D. marginatus, R. turanicus, R. sanguineus, R. bursa, R. pumilio, R. rossicus, H. asiaticum, H. anatolicum, H. scupense, H. impressum, A. persicus*, and *Ixodes* sp..

Using PCR, the infection of ticks by 11 species from 4 genera (*H. anatolicum, H. asiaticum, H. scupense, H. lumbeum, R. turanicus, R. pumilio* and others), *Rickettsia* spp., *R. heilongjiangensis*, and *C. burnetii* was determined. Ten species belonged to the family Ixodidae and one species to Argasidae. For the first time, we recorded the above-mentioned type of infection in avian ticks *A. persicus*.

OP 26

Positive RT-PCR Test Results in Patients Recovered from COVID-19 in Mauritania during the first wave of the pandemic

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The first wave of COVID-19 presented a major biosecurity challenge for Mauritania. The National Institut for Public Health Research was the only laboratory accredited to perform testing for COVID-19 by RT-PCR in our country during this public health crisis. In this study, we performed 13,021 tests during the first wave of the pandemic.

From January 20 to August 9, 2020, viral RNA was isolated from nasopharyngeal aspirates and throat swabs. Here, we were able to provide complete biosafety equipment for viral diagnostics, including the mauritanien mobile laboratory donated by the Bundeswehr Institute of Microbiology with a a viral inactivation and glove box system.

In total, we identified 2278 positive samples (17.49 %). The highest percentage of infected individuals was reported for the age group 25-44. In addition, the majority of patients was male (57 %). Compared to other countries in the African region, the mortality rate was high in Mauritania with around 2.5 %, with a median age of death at 72 years.

Overall, our data demonstrate how we could take use of biosafety equipment to perform diagnostics for a viral disease on a large scale, helping to maintain the biosecurity in our country.

OP 27

SARS-CoV-2 molecular surveillance in Georgia

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Introduction: SARS-CoV-2 genomic sequencing capacity was established at NCDC, Georgia since the beginning of COVID-19 pandemic. The aim was to monitor the circulating variants of SARS-CoV-2 in the country and share the sequenced data to the international community through GISAID.

Methods: The samples from different waves of COVID-pandemic were sequenced using Illumina next-generation sequencing platform. The PCR positive samples with Ct<28 were selected for sequencing. S gene failure on Thermo TaqPath RT-PCR

was also a criterion for sample selection throughout the pandemic. Amplicon-based ARTIC protocol sequencing was applied. The data were analyzed using CLC Genomics workbench 21.0.5. Samples with high depth and length coverage were uploaded on GISAID. As of 30 June 2023, 3213 sequences have been contributed from Georgia.

Results: Almost all variants of SARS-CoV-2 have been identified in Georgia throughout the pandemic. As the sequencing capacity strengthened over time, most of the sequenced samples (2205 strains) were from year 2022 and therefore, assigned to be omicron variants. Lately the emergence of some recombinant variants were detected which became dominant from May-June 2023.

Conclusion: Keeping surveillance on the circulating SARS-CoV-2 variants is crucial for the containment of the spreading virus. NGS met [..data lost..]

OP 28

Bacillus cereus biovar *anthracis* biofilm formation and persistence on leaves and fruits

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Bacillus cereus biovar *anthracis* (*Bc*bv*a*) is a novel anthrax causing agent infecting animals in African rainforest areas. Human infections were not yet confirmed, but exposure is likely due to hunting and consumption of 'bush meat' and was also evidenced by seroprevalence studies in humans living in affected regions.

It is still largely unknown how animals get infected with *Bc*bv*a*. One route of infection might be similar to that of *Bacillus anthracis* via spores released into the soil by dead animals. However, also monkey species living only in trees get regularly infected by *Bc*bv*a*. Thus, we assume that carrion flies are able to transmit the disease and indeed *Bc*bv*a* has been confirmed in carrion flies. These flies could potentially spread *Bc*bv*a* and its spores from infected carcasses to leaves and fruits which in turn get consumed by various monkey species.

In our study, we use *in vivo* models to show that *Bc*bv*a* is capable of persisting and even replicating on leaves and fruits as biofilm. To gain insight into biofilm formation, we use confocal laser scanning as well as electron microscopy. Multiplication and spore formation of *Bc*bv*a* are confirmed by quantification methods such as colony forming unit calculation and quantitative PCR. At last, we aim to characterize and quantify biofilm matrix composition by labeling with specific fluorescent markers and photometric measurement.

OP 29

Vector-borne disease surveillance in Polish Armed Forces

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Nowadays vector-borne diseases are common in Europe. Every year morbidity transmitted by vector infectious disease is increasing. This trend is similar in Poland. Mainly transmission of infectious diseases is being vectored by ticks, which have an impact on public health in Poland. The most significant vectored by ticks in Poland are Borreliella spp. and genus Borellia, *Anaplasma phagocytophilum*, Rickettsia spp., *Candidatus Neoehrlichia mikurensis,* and Tick-Borne Encephalitis Virus.

However, the geographical expansion of vectors can lead to the spread of diseases into new regions. Soldiers exercise in the field, participate in missions, or are stationed in Military Contingents located in different climatic conditions, which is directly related to exposure to tick-borne diseases.

Taking into account, all these aspects, it is necessary to undertake actions aimed at counteracting the effects of these diseases, and therefore, among others, to monitor vectors and the pathogens they transmit, first of all in particularly endangered areas, which in turn will become useful not only for local populations, but also for civilian employees of the army and national and allied soldiers often exercising in the field, undertaking missions, or stationed in Polish Military Contingents. Based on that in 2018 have been established vector-borne disease surveillance, which was where the military training areas, and NATO allies' force location in Poland are controlled. The surveillance assesses the prevalence of pathogens appearing in ticks collected from the 100 m^2. Based on that factor, the threat level for each location was established. The second aspect of this program is focused on the systematic dissemination of knowledge among soldiers and civil-military workers about the risks associated with diseases transmitted by ticks, prophylaxis methods, and symptoms.

OP 31

Brucella spp. contamination in unpasteurized artisanal dairy products: DNA detection and species differentiation

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Brucellosis is a worldwide zoonotic disease transmitted to humans, predominantly by the consumption of contaminated raw milk and dairy products. This study aimed to investigate the occurrence of *Brucella* spp. in 200 raw milk, ricotta, and artisan fresh cheese samples, collected from individual marketing points in four districts in Tunisia. Samples were analyzed for the presence of Brucella spp. by IS711-based real-time PCR assay. Positive samples were further analyzed by qPCR for B. melitensis and B. abortus species differentiation. The DNA of Brucella spp. was detected in 75% of the samples, *B. abortus* was detected in 31.3%, and B. melitensis was detected in 5.3% of positive samples. A percentage of 49.3% of samples co-harbored both species, while 14% of the *Brucella* spp. positive samples were not identified either as *B. abortus* or *B. melitensis*. High contamination rates were found in ricotta (86.2%), cheese (69.6%), and raw milk (72.5%) samples. The study is the first in Tunisia to assess the occurrence of *Brucella* spp. contamination in artisanal unpasteurized dairy products and showed high contamination rates. The detection of both *B. abortus* and *B. melitensis* highlights that zoonotic high-pathogen agent control remains a challenge for food safety and consumer health protection and could represent a serious emerging foodborne disease in Tunisia.

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