LUXEMBOURG
SOCIETY FOR
MICROBIOLOGY

6th LUXEMBOURG MICROBIOLOGY DAY

11 MAY 2023, 09:00 - 17:00

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Luxembourg Society for Microbiology a.s.b.l

Abbreviation: LSfM
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Welcome to the **Luxembourg Microbiology Day 2023**!

As every year, we have a very exciting day of science ahead of ourselves! The Luxembourg Microbiology Day 2023 reflects a very wide range of topics with presentations from researchers based in the country but importantly also from beyond its borders. It thereby respresents an important forum to learn and discover more about the cutting-edge research and development done by your colleagues in and around Luxembourg.
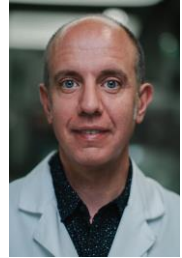
Microbiology occupies an important role in our daily lives and is central to meeting our future societal challenges, ranging from pandemic preparedness to more broadly addressing global environmental change. This is reflected in the programme of the Luxembourg Microbiology Day 2023 but also in the other activities of our society such as in our lecture series on One Earth. I warmly encourage you to engage in all of these activities, meet others and discuss science-based solutions to address these challenges.

The role which microbiology plays in society was brought into sharp focus through the COVID-19 pandemic. Luxembourg responded to this once-in-a-generation challenge through a national team effort guided by scientific evidence. Looking back over the past three years, Luxembourg had the lowest excess mortality due to COVID-19 in 2020 and 2021 amongst EU countries (plus UK) and possibly the world[1]. This demonstrates the important role which the microbiology and the boarder science community played in navigating the pandemic. However, this is not a time to be complacent as new challenges call for new microbiology-based solutions. As I already wrote last year, it is a truly exciting time to be a microbiologist!

We have many exciting activities planned this year including social gatherings. I hope to meet you on more than one occasion. The Luxembourg Society for Microbiology is **YOUR** society. If you have any suggestions for new activities, please let us know!

I hope you will all enjoy the Luxembourg Microbiology Day 2023. It is a unique opportunity to meet, exchange and come up with new ideas!

Learn and be challenged. I look forward to seeing and interacting with you.

Prof. Paul Wilmes
President
Luxembourg Society for Microbiology (LSfM)

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[1] Msemburi, W., Karlinsky, A., Knutson, V. et al. The WHO estimates of excess mortality associated with the COVID-19 pandemic. *Nature* 613, 130–137 (2023). https://doi.org/10.1038/s41586-022-05522-2

Programme

09:15	Welcome to participants and coffee
09:30	Opening speech by LSfM's President, Prof. Paul WILMES **Keynote presentation** "Together towards One Health: facts and figures of the veterinary piece of pie" by **Dr. Carole Meersschaert,** Médecine vétérinaire, Laboratoires Réunis Luxembourg SA (LU)
10:15	4 selected presentations by **PhD students** in microbiology • Refined Pipeline Approach to Isolating and Culturing Anaerobic Bacteria – **Ms Summer Bushman** (LIH, LU) • Comparison of viral concentration and RNA extraction methods for SARS-CoV-2 sequencing in raw wastewater – **Mr Ahlam Chaqroun** (LCPME, FR)
	10:45 - 11:15 Coffee break
	• Factors associated with Hepatitis E antibody titers in The Study of Health in Pomerania, Northeastern Germany (2008-2012) - **Mrs Andrea Diaz-Perez** (LIH, LU) • Biopolymer-based cryogels as a novel xero-carrier for probiotics delivery - **Mr Thierry Hellebois** (LIST, LU)
11:45	7th ordinary **General Assembly** of the LSfM
12:20	Lunch & Poster session
13:30	**Keynote presentation** "One Health goes environmental" by **Prof**. **Ana Maria de Roda Husman**, Centre for Infectious Disease Control, Laboratory for Zoonoses and Environmental Microbiology, Dutch National Institute for Public Health and the Environment (NL)
14:15	4 selected presentations by **researchers** in microbiology • Antibiotic resistance as a One-Health challenge:Role played by the environment in the circulation of Campylobacter – **Mrs Louise Hock** (LIST, LU) • The HCV-UD study: expanding access to care for hepatitis C infection among intravenous drug users in Luxembourg – **Mrs Emily Montosa Nunes** (LIH, LU) • Early detection of the non-native and potentially invasive diatom species Cymbella mexicana in Europe through eDNA metabarcoding – **Mr Demetrio Mora** (LIST, LU) • How a Gamma variant crashed the party in Luxembourg: nightlife and low immunity drive transmission of SARS-CoV-2 in summer 2021 – **Mrs Yolanda Pires-Afonso** (Health Directorate, LU)
15:15	Coffee break & Poster session
15:35	**FNR Research Funding Instruments in Luxembourg – Dr. Gideon Giesselmann**, Programme manager domain biomedical sciences (CORE), Fonds National de la Recherche (FNR, LU)
15:50	**Keynote presentation** "The newly founded ALVA (Luxembourgish Veterinary and Food Administration) and its activities in the microbiology field" by **Mr. Patrick Hau,** Ministry for Agriculture, Viticulture and rural Development (LU)
16:35	**Award ceremony** for the best oral communications (2 prizes of 250€ each) and conference achievement grants
17:00	Closing of the Luxembourg Microbiology Day & Cocktail Reception

KEYNOTE
PRESENTATIONS

Together towards One Health: facts and figures of the veterinary piece of pie

Dr. Carole Meersschaert, Médecine vétérinaire, Laboratoires Réunis Luxembourg SA (LU)

Abstract

One health is not a new concept and vets are since a long time actively participating to achieve its goals. The presentation based on personal experience as a student in veterinary medicine first, and later as a veterinary practitioner and as a veterinary laboratory biologist will go through a large panel of studies and publications realized in collaboration as well as through concrete actions and choices that have been done where the role of the vets and the veterinary laboratory is illustrated in the fight against zoonosis, against the development of antimicrobial resistance, as well as its role as sentinel through epidemiological studies.

Graduated as Doctor in veterinary medicine in 1987 (Curreghem, University of Liege) I first worked for 5 years as a companion animal veterinarian before accessing the position of scientific manager of the library of the Faculty of Veterinary Medicine for 6 years. It is during this period that I passed a Master of Science in Library and Information Management.

In 2003 I joined the veterinary laboratory environment and worked for about 12 years years in a Belgian veterinary laboratory During the last years of this position I studied at the Faculty of Medicine where I did a Master's degree in Public Health , Epidemiology and Health Economics. I work since 2016 for LR where I have been in charge of creating the first luxemburgisch private veterinary laboratory.

One Health goes environmental

Prof. Ana Maria de Roda Husman, Centre for Infectious Disease Control, Laboratory for Zoonoses and Environmental Microbiology, Dutch National Institute for Public Health and the Environment (NL)

Abstract

The One Health concept concerns a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment. When it comes to infectious diseases this is often interpreted as the zoonoses threat with the environment forgotten or boiled down to a transmission route. In my keynote I will explain the importance of fully integrating the environment into the One Health concept addressing environmental health, the environment as a surveillance tool, and the environment affecting human and animal health. Interdisciplinary research as well as the risk management framework will be promoted. Moreover, current pandemics including a future disease X and the silent pandemic of antimicrobial resistance will exemplify the importance of One Health going environmental.

Ana Maria de Roda Husman directs infectious disease research and prepares policy advise at the National Institute for Public Health and the Environment (RIVM) with over 20 years of experience. She advises policy makers at the Dutch government, the European Commission, WHO, ECDC and EFSA on the possible infectious disease risks from exposure to human pathogens in the environment, and possible intervention measures. And she was pivotal in setting up the Dutch wastewater surveillance as a human surveillance tool. From May 2012 she holds the Chair for 'Global Changes and environmental infectious diseases' at the Institute for Risk Assessment Sciences at Utrecht University. Moreover, Ana Maria has served as an expert for WHO since 2004 and is the director of the WHO Collaborating Center for Risk Assessment of Pathogens in Food and Water.

La nouvelle Administration luxembourgeoise vétérinaire et alimentaire et ses activités dans le domaine microbiologique

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Mr. Patrick Hau, Directeur-adjoint
Ministère de l'Agriculture de la Viticulture et du Développement rural
Administration luxembourgeoise vétérinaire et alimentaire

Avec l'entrée en vigueur de la loi du 8 septembre 2022, la nouvelle Administration luxembourgeoise vétérinaire et alimentaire (ALVA) a vu le jour. Elle devient ainsi la seule administration en charge des contrôles de la chaîne alimentaire, y compris les contrôles vétérinaires et contrôles des aliments pour animaux, et offre simplification et transparence au bénéfice du secteur agro-alimentaire et des consommateurs.

Sous la tutelle du ministère de l'Agriculture, de la Viticulture et du Développement rural, l'ALVA regroupe la majorité des administrations et services existants impliqués dans les contrôles de la chaîne alimentaire.

L'ALVA regroupe ainsi le contrôle des produits de la chaîne alimentaire aussi bien sur le marché luxembourgeois qu'à l'importation et à l'exportation, et

devient l'unique administration responsable des contrôles officiels concernant la sécurité et conformité de la chaîne alimentaire dans les domaines suivants :

• Contrôle du bien-être et de la santé animale ;

• Contrôle des aliments pour animaux ;

• Contrôle des denrées alimentaires ;

• Contrôle des matériaux et objets entrant en contact avec des denrées alimentaires.

Ces missions comprennent également le domaine de la sécurité microbiologique des aliments.

Les activités de l'ALVA en relation avec la sécurité microbiologique des aliments seront expliquées de même que les collaborations existantes dans ce domaine.

FNR Research Funding Instruments in Luxembourg

Dr. Gideon Giesselmann, Programme manager domain biomedical sciences (CORE), Fonds National de la Recherche (FNR, LU)

Abstract

The Luxembourg National Research Fund (FNR) is the main funder of research activities in Luxembourg. We invest public funds and private donations into research projects in various branches of science and the humanities, with an emphasis on selected core strategic areas. Furthermore, we support and coordinate activities to strengthen the link between science and society and to raise awareness for research. We also advise the Luxembourg government on research policy and strategy.

This presentation will provide an overview on the various FNR funding schemes with focus on the biomedical domain, the evaluation processes, what is new, the general role and the values of the FNR.

I work as a program manager at the Fonds National de la Recherche (FNR) in Luxembourg, coordinating research funding programs in the areas of life science, health and biomedicine. My role includes coordinating several programs such as the joint call Healthtech, the CORE program in the biomedical domain, AFR Biomedical and the international partnership ERA PerMed among others. I have a PhD with specialization in metabolic engineering and systems biology from the Saarland University.

Before joining FNR, I worked at KD pharma, a global leader in pharmaceutical omega-3 manufacturing. There, I held various positions such as IP and special projects manager, deputy R&D manager and regulatory affairs manager. I was involved in developing new products and processes, protecting intellectual property rights, managing research collaborations and ensuring compliance with regulatory standards.

ORAL
PRESENTATIONS

Refined Pipeline Approach to Isolating and Culturing Anaerobic Bacteria

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Abstract

Trillions of bacteria reside in our gut, making up our gut microbiome. We depend on these bacteria to maintain homoeostasis, but they also seem to play a role in the development and progression of diseases, such as multiple sclerosis, type 1 diabetes, and inflammatory bowel diseases1-4. The underlying mechanisms of how bacteria contribute to the development of diseases is largely unknown, but their activity can be modulated by factors such as one's diet5-6. Given that bacteria can be modulated through external manipulation, targeted microbiota modulation to control diseases symptoms or even prevent disease is the ultimate goal. To establish this therapeutic method, further characterization of the microbiome and isolation of individual bacterial strains are needed.

In order to better understand the complex interactions that occur between gut microbiome members, it is important to first study them in a simplified in vitro system to characterize the functionalities of each bacterial strain. However, finding the optimal growth conditions for specialized bacterial species, especially for anaerobic microbiome members, can be challenging. Since this is the first step in establishing a complex multi-strain community for use in further in vitro and in vivo experiments, our goal is to find the optimal growth conditions specific to single strains that are part of the human gut microbiome. The resulting media can then be used not only to isolate individual strains of interest from fecal samples in a more targeted and rapid manner, but also to combine knowledge of the individual strains to develop a broad spectrum medium that meets the combined needs of a complex bacterial community.

Our developed pipeline allows us to test and establish both liquid media and agar media plates which isolate individual strains, as well as grow and study complex bacterial human gut microbiome communities.

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Comparison of viral concentration and RNA extraction methods for SARS-CoV-2 sequencing in raw wastewater

Ahlam Chaqroun[1][*][a], Ghina El soufi[2][a], Julie Loutreul[3], Zuzana Gerber[4], Jean-François Deleuze[4], Obépine consortium, Christophe Gantzer[1], Olivier Rohr[2], Nicolas Boudaud[3], Clémentine Wallet[2][b], Isabelle Bertrand[1][b]

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Abstract

The circulation of SARS-CoV-2 in the human population and its genetic evolution monitoring are evaluated from wastewater (WW) using molecular methods which are mostly performed on small volumes of WW (Ahmed et al.,2022). This was useful during high viral prevalence in the population. However, the level of the virus circulation can be very low due to both epidemic fluctuations and early stages of variant emergence. Therefore, it is critical to improve methods for sensitive detection and a high-quality sequencing.

As part of EmerEaUde ANRS project, the aim was to define suitable virus concentration and RNA extraction methods for the identification of emerging viruses in WW by sequencing to face future viral emergence.

First, three laboratories have compared two RNA extraction protocols on non-concentrated WW (5 mL), using in common a commercial protocol A based on silica columns and specific for WW. Protocols B and C used the same commercial protocol based on silica beads, with a neutral phenol-chloroform treatment (PCT) for protocol C. Protocol D was a homemade protocol using silica beads and acidic PCT. Each laboratory analyzed nine WW samples in quintuplet (n=45 analysis per laboratory). The RT-qPCR assays targeted the E gene. Evaluating both RNA quantities and removal of RT-PCR inhibitors, protocols A and C were the most efficient (Mann-Whitney, p-values < 0.05). Secondly, larger volumes of WW (100-500 mL), were analyzed using two ultrafiltration methods systematically combined with neutral or acid PCT. As expected, the analysis of the highest volumes of WW led to the recovery of the highest quantities of RNA. Next-generation sequencing analyses are in progress using the Illumina COVIDSeqTM (ARTIC v4.1) library preparation and NovaSeq 6000 SP Reagent Kit v1.5 (300 cycles) short-read sequencing (Gerber et al., 2022) to determine the most reliable concentration/extraction protocol for sequencing from WW.

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Factors associated with Hepatitis E antibody titers in The Study of Health in Pomerania, Northeastern Germany (2008-2012)

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Abstract

Hepatitis E (HEV) incidence showed a stepwise increase in Mecklenburg-Pomerania between 2008 and 2012, with estimates above the national mean since 2009. Genotype 3 causes autochthonous cases in Germany, although humans are susceptible to five of the eight genotypes. Various animal species are susceptible to HEV G3, but pigs and wild boar are the main reservoirs. The main transmission pathway seems to be through pork/boar meat consumption. However, recent studies have shown that direct and indirect contact with susceptible species are additional risk factors. The transmission pathways to humans are poorly defined, presumably because complex diseases such as HEV are analytically challenging.

Using data from the Study of Health in Pomerania, we analyzed the association between HEV IgG antibody titers and different risk factors such as meat consumption and animal contact. We used quantile regression to reduce the loss of power due to dichotomization of the outcome and compared results to those of logistic regression. Additionally, we introduced in the models an interaction term to determine if the effect of the risk factors differed across spatial settings. To reflect on the robustness of our findings, we performed asensitivity analysis using a sequence of percentiles in the quantile regression models.

The estimated seroprevalence was 27.26% (95% CI 25.95, 28.62%). Age was directly associated with HEV status in all logistic regression models (OR 1.02; CI 1.02, 1.03). In urban settings, participants with contact to domestic carnivores (dogs, cats) had higher antibodies (12.76 U/ml; CI 0.25, 25.27) compared to participants without such contact. Participants in rural coastal areas had higher antibody titers for high meat (14.50 U/ml; CI 3.85, 25.147) and sausage consumption (14.48 U/ml; CI 4.08, 24.88) than participants in these areas with low consumption. In the sensitivity analysis, the regression coefficients of the statistically significant interactions maintained a positive trend.

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Biopolymer-based cryogels as a novel xero-carrier for probiotics delivery

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Abstract

An incrementing number of studies have pinpointed the influential role of gut microbiota on human health from innate immunity to chronic inflammatory disease (e.g., cardiovascular disease, diabetes, several forms of cancer etc.) to appetite regulation and energy metabolism. Probiotics are defined as human gut relevant commensals and microbes that have generic or core effects on gut physiology and homeostasis or that support the health of the reproductive tract, oral cavity, lungs, skin and gut-brain axis. (Hill et al., 2014). The administration of probiotics to the human host is commonly achieved via the ingestion of either functional foods (e.g. fermented foodstuff) or food supplements. The encapsulation of probiotics i.e., the physically- or chemically-induced engrafting of living cells in biopolymer structure-engineered micro-scaffolds has gained much popularity in the last two decades thanks to its potential to hamper the sublethal effects of extrinsic stressors (i.e., exposure to mechanical stress, heating, low pH, oxygen, water vapour, high ionic strength, peptic cleaving enzymes, bile salts, etc.) associated with food processing, storage, and gastrointestinal transit stimuli. (Yao et al., 2020). The present study aimed to assess the ability of milk protein/dietary fibre-based cryogel monoliths as novel encapsulation xero-scaffolds to preserve the biological activity of an EFSA approved probiotic strain i.e. Lacticaseibacillus rhamnosus GG (LGG) cells. In view of this, the viability of LGG throughout cryogenic processing, controlled storage conditions and in vitro digestion was monitored.

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Acknowledgements

Antibiotic resistance as a One-Health challenge: Role played by the environment in the circulation of Campylobacter

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Abstract

With 4.95 million deaths associated with drug resistant bacterial infections in 2019, the antimicrobial resistance (AMR) is considered the new silent pandemic [1]. The unreasonable use of antibiotics in humans and farm animals has increased the selection of resistant bacteria making them well-known hotspots of AMR. However, the transmission and spread of bacteria carrying AMR genes in wildlife and environment could represent an additional challenge by turning the environment into an extra AMR hotspot. Among AMR bacteria considered as high concern for public health, Campylobacter is the leading bacteria of human food infection in European Union (EU) since 2005 [2-3]. This study aims to assess the prevalence of AMR in Campylobacter isolated from surface water and wild birds in Luxembourg to draft a picture of AMR carriage in a One Health approach.

Luxembourg presents one of the highest incidences of Campylobacter gastroenteritis infections in the EU [3], with 62 and 82% of human C. jejuni and C. coli resistant to at least one antibiotic. In comparison, only 22% and 3% of resistant C. jejuni and C. coli reach surface water and the survival of C. jejuni is low. In contrast, wild birds are mainly contaminated by C. jejuni including 12% of isolates resistant to at least one antibiotic and displaying a different AMR profile than human isolates. These phenotypes are concordant with AMR genotypes except for beta-lactam and aminoglycoside resistances for which genes and mutations are present without the expression of a resistant phenotype. Multi-resistant isolates were detected in water downstream a wastewater treatment plant. Consequently, the role played by the environment in the circulation of AMR bacteria and genes is non-negligible and a One-Health surveillance is required to guide public health policies in the control of foodborne illness.

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The HCV-UD study: expanding access to care for hepatitis C infection among intravenous drug users in Luxembourg

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Abstract

Intravenous drug users (IDU) represent the majority of new cases of HCV infection in Luxembourg. We established an interventional program in four different harm reduction centers to improve screening and medical care of IDU infected with HCV. Participants were offered blood drawing for HCV, HIV, HBV and syphillis serologies, viral loads, HCV genotyping, liver biomarkers and a fibroscan. Interviews were conducted using a standardized epidemiological questionnaire. 480 participants (72.9% male) were recruited between October 2015 and December 2019 and were followed until December 2021 for Direct Acting Antiviral (DAA)'s treatment outcome. Among them, 74% were unemployed, 48.4% had no income, and 71.2% reported to live in unstable housing or were homeless. 58.6% of the participants were under Opioid Substitution Therapy, 49.1% were injecting drugs every day, 59.2% had heavy alcohol consumption, 33.2% had been incarcerated at least once, and 21.1% of women were sex worker. Among the 473 participants tested for HCV serological markers, 71% (336) were anti-HCV positive, 64.3% of those (216) were HCV RNA positive. Four genotypes were identified (56% G1, 0.5% G2, 38% G3, 5.5% G4) in 203 HCV sequences. 11.2% were HIV positive, and 1.5% were HBs Ag positive. 72 drug users (33.3% of HCV RNA positive cases) initiated a DAA treatment at enrolment: 84.7% achieved Sustained Virological Response 3 weeks after the end of the treatment (SVR3), and 73.6% SVR12 when retained in care. Reinfections were recorded in 11.5% of the cases or 2.2 reinfections/100 patient-years of follow-up. Phylogenetic analyses revealed 18 clusters with more than 5 sequences and a large G1a cluster of 40 sequences indicating frequent transmissions across long intervals of years. In conclusion, the study demonstrated the feasibility of effective screening and HCV care among high risk drug users in harm reduction sites to accelerate HCV micro-elimination in this key group.

Early detection of the non-native and potentially invasive diatom species *Cymbella mexicana* in Europe through eDNA metabarcoding

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Abstract

The distribution of non-native species and their effects to ecosystems have been extensively studied in macroorganisms. However, this has not been the case for microbes, although their disruptive effects can be broader to the ones caused by large organisms. Recently, there have increasing reports about the spread of non-native microorganisms such as microalgae around the globe. This has been mainly attributed to habitat changes due to human activities and climate change. In addition, novel methods applied to environmental DNA (eDNA) have transformed the way microbial communities are analysed, allowing rapid identification from environmental samples, e.g., water, soil, or air. As part of current projects aimed at characterising microalgal and cyanobacterial communities through DNA-based methods in large rivers, we report the first occurrences of the diatom species Cymbella mexicana in Europe, in the rivers Moselle, Spree and Lahn, a species native to Central and North America. We initially detected this species by eDNA metabarcoding. To exclude the probability of false positive detections, we refined the taxonomic assignment under a phylogenetic framework. Additionally, examinations by light and scanning electron microscopy further confirmed the identity of this species. Given the biology of this microalgae, able to produce large mucilaginous stalks, it has the potential to turn into a nuisance species as its two sister species Cymbella janischii (reported in 2022 as invasive in Japan), and the highly invasive Didymosphenia geminata, which form extensive blooms that cover riverbeds completely, leading to major ecological and economic impacts. Our study highlights the complementarity of different methods (e.g., metabarcoding, phylogenetics and microscopy) in the early detection of this non-native species, setting the baseline for monitoring its spread and potential impacts in freshwater ecosystems across Europe.

How a Gamma variant crashed the party in Luxembourg: nightlife and low immunity drive transmission of SARS-CoV-2 in summer 2021

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Abstract

During the summer 2021, while the Delta variant was predominant elsewhere in Europe, Luxembourg experienced a large outbreak with a Gamma variant of SARS-CoV-2. This occurred in mid-June shortly after digital COVID-19 certificates were introduced and nightlife restrictions were relaxed before national holiday festivities. This provided us with a unique opportunity to investigate molecular and epidemiological factors contributing to SARS-CoV-2 transmission.

All laboratory confirmed cases of SARS-CoV-2 were interviewed by contact tracing teams. Exposure information on attendance of nightlife venue, family gatherings, schools and workplaces was recorded. Whole genome sequencing was performed on random SARS-CoV-2 samples and sequences were submitted to GISAID. We investigated infection clusters by combining phylogenetic with epidemiological exposure data, in particular certain nightlife venues.

Between 24th June and 12th August 2021, 1149 cases of the Gamma variant P.1.17.1 were identified in Luxembourg's surveillance system, representing 39.2% of all sequenced cases. In the first 2 weeks of the outbreak, nightlife was the most likely source setting (47.9%), with cases having a median age of 26 years (51% male). Family (46.7%) and workplace (12.9%) settings became more predominant in the last four weeks of the outbreak when the median age increased to 31 years (p<0.05). The presence or absence of the C26645T SNP in the M protein was associated with attending specific nightlife venues. Overall, 82.9% of the cases were unvaccinated. While some cases of P.1.17.1 were also detected in neighbouring countries, 75.6% of all sequences submitted to GISAID originated from Luxembourg, making it a major hub of this variant.

Our study shows that nightlife activities in combination with low population immunity contributed to substantial transmission of SARS-CoV-2 among young adults during summer 2021. Our study illustrates how routine sequencing in combination with contact tracing may assist in identifying high-risk transmission settings and thus informing outbreak investigations.

POSTER PRESENTATIONS

Transcriptomic analysis of *Clostridioides difficile* behaviour in presence of bacterial neighbours

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Abstract

Most cases of nosocomial infectious diarrhea are due to Clostridioides difficile, an anaerobic, gram-positive spore-forming bacterium responsible of pseudomembranous colitis. However, some people do not develop symptoms despite the presence of the bacterium in their feces. This could be due to their microbiome which could influence Clostridioides difficile behaviour (Martinez et al. 2022). Risk factors associated with infection are firstly a dysbiosis that would allow a better gut colonization by Clostridioides difficile, moreover, antibiotics, proton inhibitors as well as age and recent hospitalization also enhance Clostridioides difficile colonization. (Anjewierden et al. 2021)

The objective is to highlight changes of Clostridioides difficile gene expression with and without bacterial neighbours at 12hours by metranscriptomic.

The experimental design was based on transwell experiments where mRNA from Clostridioides difficile in different growth conditions (with and without microbiome) have been collected at different times (12h and 24h) in order to carry out a metatranscriptomic analysis.

A paired comparison between condition showed that 12.17% and 13.63% of Clostridioides difficile genes had a significant change of expression in presence of two different feces at 12hours. By the enrichment analysis, the Stickland metabolism as well as virulent process showed a significant expression change in presence of feces.

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Development of immunotherapeutic complexes eliciting complement activation towards multidrug-resistant *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa (P. aeruginosa) is a Gram-negative opportunistic bacterium causing a variety of life-threatening infections in immunocompromised patients. As a new approach to combat bacterial infections, we developed complement activating multimeric immunotherapeutic complexes (CoMiX) using the C4bp multimerising scaffold to activate complement on targeted bacteria. We combined on the C4bp scaffold a single-chain fragment variable (scFv) recognising the exopolysaccharide (Psl) matrix of *P. aeruginosa* to either an Fc-dimer inducing the complement classical pathway (CoMiX-Fc) or Factor H-related protein 1 (CoMiX-FHR1) competing with FH binding and eliciting the complement alternative pathway.

The binding of CoMiX was tested on reference strains and 29 antibiotic-resistant clinical isolates retrieved from sputa of cystic fibrosis patients or from tracheobronchial aspirates of subjects housed in medical Intensive Care Units (ICU) by whole cell ELISA. The anti-Psl CoMiX bound to ~80% of clinical isolates. CoMiX-Fc and CoMiX-FHR1 significantly increased C3b and C5b9 deposition (p<0.001) on clinical isolates and reference strains as compared to controls. Supplementing with 20 to 50% human serum with 3 µg of CoMiX-Fc or CoMiX-FHR1 resulted in a dose-response inhibition of the growth of luciferase-expressing PAO1 strain (PAO1-Luc) during 5 hours compared to serum alone as well as in a 30-50% (p<0.05) reduction for the clinical isolates in the number of Colony Forming Units (CFUs)/plate compared to serum alone. Preliminary data in mice infected with the PAO1 strain showed an improvement of overall survival and weight loss in comparison to the controls during the disease course using a single dose of CoMiX-Fc.

In conclusion, we have developed new immunotherapeutic complexes having a direct killing effect on *P. aeruginosa*.

Harmful algal blooms in Luxembourg bathing waters: how to boost data collection and improve public health risk assessment?

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Abstract

Representative monitoring of harmful cyanobacteria bloom (CyanoHAB) duration, intensity, and timing are crucial for the provision of timely warnings to protect public health. It further plays a crucial role in understanding short and long-term trends in cyanobacteria bloom dynamics, especially in a context of climate change. Unfortunately, high resolution datasets accounting for the large scales of variation in CyanoHAB spatiotemporal dynamics are often lacking. To boost data collection, we have been implementing innovative technological solutions spanning from remote and *in situ* sensing to automated image acquisition and rapid *in situ* cyanotoxin dosage. Because each tool has a different spatiotemporal coverage and displays specific advantages and limitations, we identify those that are best suited for operational monitoring of CyanoHABs, with a focus on the Upper-Sûre reservoir. The latter waterbody provides 50% of the drinking water demand in Luxembourg and it is the largest recreational area with 6 official beaches. CyanoHABs are reported in the Upper-Sûre reservoir since the 1970s and their yearly recurrence from late summer to fall impacts ecosystem health and poses a health risk for lake users. Besides technology-driven innovation, we aim at fostering active involvement of citizens into the process of risk assessment and monitoring. Such participatory approach is involving lake users (bathers, fishermen, divers) through participatory near real-time reporting of CyanoHAB using a mobile app and through rapid *in situ* testing for the occurrence of cyanotoxins in water. Altogether, these initiatives help increasing the spatiotemporal coverage of field data while also raising environmental awareness. The data acquired by the technology and citizen-driven approaches not only aim at reducing the need for labour-intensive field sampling and laboratory analyses, but they will also feed models for accurate forecasting of CyanoHABs and for the design of reliable early warning systems towards optimal management and control of public health risks.

Presence of human enteric and respiratory viruses in wastewater during the COVID-19 pandemic: a surveillance study

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Abstract

Wastewater-based epidemiology (WBE) has been used successfully to monitor the circulation of SARS-CoV-2 in communities. However, its application for most other respiratory or enteric viruses of public health concern is still under evaluation. In that context, we performed a retrospective WBE study to understand the circulation of a panel of respiratory and enteric viruses in communities and to determine whether the SARS-CoV-2 pandemic had an impact on their circulation. Four wastewater treatment plants in Luxembourg, covering 52% of the population were sampled from March 2020 to March 2023. We quantified the RNA concentrations of noroviruses (NoV) genogroup I (GI) and GII, human enteroviruses (hEntV), influenza A virus (IAV), human respiratory syncytial virus (hRSV) and human seasonal coronaviruses (hCoVs; i.e. hCoV-229E, hCoV-OC43, hCoV-NL63, hCoV-HKU1), using quantitative RT-PCR. We also compiled available data of laboratory confirmed human cases of NoV, hRSV and IAV in Luxembourg over the same time period. Such data were totally missing for some other targets (e.g. hEntV and hCoVs).

We detected RNA from all tested viruses in wastewater. NoV GI (94.8% positive samples) and GII (89.5% positive samples), hEntV (86.7% positive samples) and hRSV (27.7% positive samples) were the most common viruses found over the entire period analysed. HCoVs (18.9% positive samples), as well as IAV (1.8% positive samples) were also found to a lesser extent. We observed temporal fluctuations for each virus, suggesting that the public health measures for the SARS-CoV-2 pandemic affected their circulation. We also found associations between viral RNA concentrations in wastewater and reported human cases for influenza and hRSV.

Our study demonstrates the feasibility and usefulness of WBE for monitoring viral respiratory and enteric infections in communities, especially when comprehensive surveillance in the population itself is lacking.

Indoor microbiology in Environmental Medicine – a new tool for healthcare

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Abstract

The economic burden of respiratory diseases is one of the heaviest in Europe's health services, mainly due to its chronic nature, frequent need for hospitalization, and costly management. The indoor environment has been associated with triggering and/ or exacerbation of symptoms in many clinical conditions. Identifying the presence of triggers and acting upon the improvement of the indoor conditions, represents a new tool to manage respiratory disease, in collaboration with clinicians. Since November 2022, the new laboratory for the "Built Environment" in the LNS has become operational. The Lab carries out analyses following medical prescriptions by general practitioners or specialist medical doctors. This may be, for example, persisting respiratory complaints or infections and investigations in immune-suppressed patients e.g. cancer, transplantation. We currently focus on bacteria, fungi and allergens.

The Lab receives air samples and proceeds with the quantification of both bacteria and fungi in the indoor air, and with the identification, by optical microscopy, of the fungi found in the air in relevant levels. Whenever there are visible fungi on the houses' surfaces samples are also collected for their identification.

As an example, our first results, based on 20 houses and 262 mould samples analysed, show that the indoor presence of moulds was on average 203 UFC/m3 (SD 330) ranging from 4 to 1828 UFC/m3. Comparing to the outdoor, used as reference, we have an average of 190 UFC/m3 (SD 196) with a range between 24 and 732 UFC/m3. Indoor higher levels of moulds were frequently associated with the prevalence of toxigenic species (*Penicillium sp., and Aspergillus sp.).*

Overall, our results clearly point out the need to further develop methodologies that will allow us to make a profound characterization of the Luxembourgish built environment. As such, we can build capacity that is targeted at the specific medical needs in Luxembourg.

An innovative training programme focusing on wastewater surveillance

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Abstract

Wastewater-based epidemiology (WBE) has come to the fore since the Covid-19 health crisis. Indeed, WBE has many advantages over classical epidemiological techniques, such as a rapid data collection, a non-invasive and non-discriminating sampling representing a pool of a global population, and an ability to target a specific area or recreational events. The reduction of sanitary measures related to Covid-19 and thus the decrease in the direct monitoring of infected individuals (Covid law of 1st April 2023 in Luxembourg) underlines the important role of wastewater analysis to ensure disease surveillance as a powerful asset for public health. The major weakness of WBE remains the lack of standardisation and harmonisation of methodologies used for both sample collection and data analysis. The European ERASMUS+ project, called OneHealthWatch, can help to tackle this issue by developing an innovative training programme focusing on wastewater surveillance. Still in its preliminary stages, the project aims to define and disseminate best practices in wastewater monitoring to enable rapid implementation of safety measures and contingency procedures in case of alarming detection such as the Covid-19 pandemic, and to harmonise and standardise sampling and analysis methods at national and then European level. The platform is intended for a wide range of audiences, from professionals working in the field of wastewater to health professionals and the general public. The main challenge in developing such an e-learning platform is the ability to share and adapt scientific, reliable, accurate and up-to-date content in a didactic and entertaining way. Who hasn't had to follow a monotonous and boring training course? It is with this experience in mind that the platform is built to create clear and adapted content for WBE actors as well as for other neophytes to raise awareness on the major role that wastewater monitoring will play in the future.

Seeing is Believing: MALDI Imaging Provides Unprecedented Molecular Detail of Microbial Interactions

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Abstract

Microbial interactions play a critical role in shaping our world, yet studying them in their natural environment has been a challenge. Traditional methods rely either on cultivation, which alters the microbial interactions, or extraction, which provides limited spatially resolved information. MALDI imaging, a cutting-edge technique, already applied for clinical research purposes, could provide a new way to study in situ microbial interactions with an unprecedented detail and resolution[1]. The aim of this study was to demonstrate the feasibility of using MALDI imaging, at atmospheric pressure, to study the growth of microbial colonies. To achieve this, bacterial strains were cultured overnight on filter membranes and transferred on a stainless-steel plate. The latter were then subjected to MALDI analysis on a high-resolution mass spectrometer to generate an image of metabolites, with a spatial resolution of 25 µm per pixel. Resulting full scan and targeted MS/MS mass spectra, enabled the acquisition of molecular profiles, the spatial localisation of 995 microbial mass-to-charge ratio values, as well as the structural information of several molecules within the 200-2000 Da range. A total of 248 bacterial metabolites, including quorum sensing molecules, and lipids were annotated by using the LipostarMSI platform. Finally, a super-resolution image reconstruction, based on an artificial intelligence approach, was performed to enable metabolites visualisation at a higher resolution than the current hardware allows (5 µm/pixel in our current setup). Thus, combination of such spatial omics technology with other multimodal imaging or omics techniques, and artificial intelligence, might revolutionize our understanding of microbial growth and interactions with their hosts directly within complex environments (e.g., biofilm).

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Acknowledgements

Stabilisation and preservation of *Lacticaseibacillus rhamnosus* GG in Spirulina (*Arthrospira platensis*) protein isolate-based lyophilisates

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Abstract

Anhydrobiotics, i.e. structurally engineered xero-carriers conveying living cells are extensively used in the production of probiotic supplements. As a mimimum requirement, the probiotic xero-carriers should preserve the biological activity of the living cells under common physicochemical stressors such as low pH, high temperature and ionic strength, exposure to water vapour and oxygen, bile salts, etc., encountered during processing, storage and gastrointestinal transit (Seifert et al., 2019). Milk proteins are considered as the golden standard for preserving the biological activity of a broad range of probiotic bacteria including Lactobacilli and Bifidobacteria. Due to dietary, socio-cultural and ecological constraints, i.e., allergenicity, high carbon footprint or the exclusion of specific consumer groups, the demand of plant-based protein sources is growing. An excellent alternative for milk proteins is microalgal proteins, e.g., derived from Spirulina or Chlorella, due to their high protein and phytochemicals content, biological value, sustainable and eco-friendly character (Acquah et al., 2021). In the present work, the impact of Spirulina protein isolate on the survival rate of *Lacticaseibacillus rhamnosus* GG (LGG) during lyophilization, storage and in vitro digestion was investigated. Different approaches in structuring the xero-carrier precursors i.e., solution or hydrogel prepared via direct or indirect (fermentation) acidification, were assessed. As a comparison, pea and whey protein isolate-based lyophilised templates were also tested. Accelerated storage trials at different temperature and water activity) conditions were conducted for modelling the LGG cell inactivation kinetics. Finally, the biological activity of LGG as associated to the colloidal changes of the delivery systems were analysed under simulated in-vitro digestion conditions.

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Interest of advanced molecular biological tools to identify the rise and fall of SARS-CoV-2 variants in Luxembourg wastewater.

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Abstract

Surveillance of SARS-CoV-2 in wastewater is a promising tool for epidemiological surveillance, as it correlates viral RNA levels with population dynamics of infection and viral diversity. However, the complex mixture of viral lineages in wastewater samples makes it difficult to track specific variants or lineages circulating in the population. We retrospectively analysed sewage samples from the 3 largest sewage treatment plants in the country to identify specific signature mutations of the different SARS-CoV-2 lineages and to estimate their relative abundance in the sewage. These abundances were then compared to those observed in the clinical genomic surveillance of infected persons between March 2020 and December 2023. Two distinct genomic approaches were used and compared, namely digital droplet RT-PCR and high throughput sequencing. The results indicated that both methods are appropriate for estimating the distribution of variants of concern from wastewater, although RT-ddPCR is more sensitive for detecting the presence of circulating variant of concern at low relative abundances. NGS and RT-ddPCR results showed similar dynamics of circulating major variants. Altogether, the results showed that several variants of concern emerged throughout the pandemic period, became dominant and were replaced by the next variant at different time points during the study. In conclusion, we have demonstrated the reliability of wastewater surveillance, both by NGS and RT-ddPCR, for monitoring the rise and fall of SARS-CoV-2 variants, increasing the set of epidemiological tools to monitor SARS-CoV-2 diversity. Our results highlight the potential use of wastewater samples for genomic surveillance also, allowing for comprehensive tracing of circulating lineages, emergent variants and specific mutations, especially when clinical surveillance is scaled down. Our approaches have the potential to be expanded beyond SARS-CoV-2, to other viruses prevalent in wastewater and for which clinical surveillance is limited.

Zero-dimensional modelling and bacterial characterization of an aerobic granular sludge reactor

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Abstract

Background: Aerobic granular sludge (AGS) has emerged as a novel wastewater treatment technology and as a suppressing alternative to conventional activated sludge. The development of mathematical models of the AGS process, which can be used for wastewater treatment plant (WWTP) design and optimization, is therefore necessary to support successful implementation of AGS technology. This study aims to develop a zero-dimensional (0D) AGS model that can be used in engineering practice for the above-mentioned purposes.

Results: A laboratory AGS sequencing batch reactor (SBR), removing soluble organic substrate, nitrogen and phosphorus, was fed with artificial wastewater and modelled using a 0D approach. Model development was supported by bacterial characterization using 16S rRNA gene amplicon high-throughput sequencing. The mathematical model was based on the activated sludge model no. 2d (ASM2d) and extended with both two-step nitrification as implemented in the wastewater treatment plant simulator Sumo19 (Dynamita SARL) and ammonia nitrogen adsorption and desorption according to the Langmuir model. The variations of ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, orthophosphate phosphorus and dissolved oxygen concentrations during one cycle of the SBR were successfully reproduced by the model (all Pearson's r values ≥ 0.93 and all r2 ≥ 0.86).

Conclusions: The 0D modelling approach was proven to be applicable to AGS. It is possible that the 0D approach can fill the gap that has developed between engineering and research in the biofilm modelling community. The 0D modelling approach therefore merits further exploration using reactors fed with real wastewater, as well as on pilot and full-scale WWTPs.

ExpoBiome: an integrative approach to study the human gut microbiome

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Abstract

The human gut microbiome is a complex ecosystem that is tightly linked to human physiology, health, and disease. Several chronic diseases display functional and compositional changes to the microbiome. Studies focusing on the taxonomic composition of the gut microbiome have already reported negative associations between microbial diversity and features of chronic diseases. Recent studies have also reported the role of microbiome-derived effector molecules in organ, mucosal and immune functions. Particularly, there exists a tight and stable interconnection between the immune system and the gut microbiome, which can become dysregulated in diseases with inflammatory signatures, such as Parkinson's disease (PD) or rheumatoid arthritis (RA). For example, studies in germ-free murine models demonstrate that intestinal colonisation aggravates the symptoms of PD, highlighting the importance of the gut-brain axis in neurological diseases. Nevertheless, the complex of microbiome-derived molecules is vast and its role in mediating microbiome-immune system interactions is poorly understood. Hence, the key question facing the field is whether microbiome differences are cause or consequence of disease. Such knowledge is necessary to predict and control microbiome-linked health and disease.

Here, we present ExpoBiome, an integrative approach that focuses on exposure to the "environment within", i.e., the gut microbiome with its produced molecular complex, to resolve how changes to the gut microbiome manifest themselves functionally and impact disease processes. Using contextualised prior knowledge in the ExpoBiome Map (expobiome.lcsb.uni.lu/), microbiome multi-omics data and machine learning methods, we are aiming to identify microbial molecules associated with PD- and RA-specific immunophenotypes. To allow disease-relevant predictions, we follow the signatures of identified microbiome-derived molecules longitudinally during a microbiome-targeted intervention (therapeutic fasting) in PD and RA patients. The insights generated through ExpoBiome will not only expand the knowledge on the PD- and RA-associated microbiome, but importantly will generate critical information for elucidating the mechanistic role of the microbiome molecular complex in human health and disease.

7ᵉ Assemblée Générale Ordinaire de la LSfM

7e Assemblée Générale Ordinaire de la LSfM
11 Mai 2023 (11:45) au LNS, Dudelange

Ordre du Jour

i. **Allocution par M. le Président, Prof. Paul WILMES**

ii. **Bilan des adhésions à la LSfM et des activités pour l'exercice 2022 par Mme Conny MATHAY, Secrétaire Général**

iii. **Bilan des comptes de la LSfM pour l'exercice 2022 par Mme Lorieza NEUBERGER-CASTILLO, Trésorier**

iv. **Compte-rendu sur le bilan des comptes de la LSfM par les réviseurs de caisse de l'exercice 2022, Mme Ines KOZAR et Mme Emilie MULLER.**

v. **Approbation des bilans d'activité et des comptes de la LSfM pour l'exercice 2022 par l'Assemblée Générale**

vi. **Désignation des Réviseurs de Caisse pour l'exercice 2023**

vii. **Validation des cotisations pour l'exercice 2023/2024**

viii. **Renouvellement et élection des candidatures pour le Conseil d'Administration de la LSfM**

ix. **Prévisions budgétaires pour l'exercice 2023, par Mme Lorieza NEUBERGER-CASTILLO, Trésorier**

x. **Présentation des activités de la LSfM pour l'exercice 2023, par Mme Conny MATHAY, Secrétaire Général**

xi. **Questions et sujets divers, discussion générale**

xii. **Clôture de l'Assemblée Générale par M. le Président, Paul WILMES**

Notes

Notes

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📍 **5 Apr - Piotr Oleskowicz-Popiel**
Poznan University of Technology
Resource recovery from waste - a short story on open culture fermentation

📍 **3 May - Tim Lenton**, *University of Exeter*

📍 **7 Jun - Jack Gilbert**, *University of California San Diego*

📍 **5 Jul - Donato Giovannelli**, *University of Naples*

📍 **6 Sep - Marie-Agnès Jacques**, *INRAE*

📍 **4 Oct - Hélène Carabin**, *Université de Montréal*

📍 **8 Nov - W. Ford Doolittle**, *Dalhousie University*

📍 **29 Nov - Rita Colwell**, *University of Maryland, College Park and Johns Hopkins University Bloomberg School of Public Health*

For more information

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