Microbiome Network Meeting + BBQ

July 20, 2022

Veterinarium Progressum, Oertzenweg 19b, 14163 Berlin (Düppel)

ILLUSTRATION BY ANTOINE DORÉ

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Microbiome Network Meeting

Dear participants,

Welcome to the first Microbiome Network Meeting + BBQ on July 20th 2022, at the Veterinarium Progressum. This event is organized by young researchers from universities and research institutes in and around Berlin, and by the FoodBerlin research network (www.foodberlin.de). It is our pleasure to share this event with all of you.

This initiative aims to promote a multidisciplinary network around the exciting world of microbiome research in Berlin and its surroundings, everything in a relaxed environment. We planned a half-day event with two fantastic keynote speakers, Sofia Forslund and Stefan Jordan, and all of you, early career researchers, to share knowledge and experience through talks, posters and a science slam. We hope this event promotes the exchange of experiences and brings together researchers for current and future works.

We understand the difficulties due to the current pandemic situation; however, we hope to have as much interaction as possible. Due to the rising number of cases, we encourage you to wear masks during the inside parts of the event.

We thank our sponsors for their support and all of you for your interest in the event. We wish the event could be as helpful and exciting for you as we expected.

Your Microbiome Network Meeting organizing committee

Program

12.30 - 13.00h	Arrival, registration, putting up of posters
13.00 - 13.10h	Welcome and introduction
13.10 - 13.50h	Sofia Forslund - *Host and microbiome in the context of cardiovascular disease and its treatment* (30 min + 10 min discussion)
13.50 - 14.30h	Flash talks session I (5 min w/ppt or flipchart) + 3 min discussion

1. Selina Schmidt (FU) - *Effect of biocides on the evolution and spread of resistance*
2. Ankur Midha (FU) - *The microbiome of the intestinal parasitic nematode Ascaris suum is derived but distinct from its host*
3. Sonja Wende (ZALF) - *Functional Metagenomics as a tool to decipher crop microbiomes*

14.30 - 16.00h	Posters and coffee/cake
16.00 - 16.40h	Stefan Jordan - *Microbiome, Metabolism, Inflammation: Dietary Energy in Immunoregulation and Inflammatory Disease* (30 min + 10 min discussion)
16.40 - 17.20h	Flash talks session II (5 min w/ppt or flipchart) + 3 min discussion

4. Hendrik Bartolomaeus (ECRC-MDC/Charité) - *In-depth spatial analysis of microbiome-immune interaction in mice*
5. Tobias Goris (DIfE) - *Transformation of flavonoids by human gut bacteria*
6. Rebecca Luise Knoll (ECRC-MDC/Charité) - *Patterns of gut microbial dysbiosis despite probiotic supplementation at day 28 of preterm life*
7. Jannike Krause (DRFZ) - *Sample logistics affect structural and functional profiles of faecal microbiota*

17.45 - 18.15h	Science slam hosted by Ankur Midha (7 min - w/ppt or own props)

- Lisa Budzinski - *Horospoop*
- Bruno Rocha Cordeiro Costa - *Wildling microbiota induces changes in the immune and structural cell compartments of the skin*
- Nizar Shayya - *Colonization Resistance: a first line of defense*

18.15 - 18.45	Microbiome Pub Quiz
19.00h	Open Grill
20:45h	Award ceremony and closure of the event

Poster session and flash talks

Po-1: Anna Fagundes - *Respiratory tract microbiota modulates type 17 immunity and homeostasis of non-hematopoietic cells*

Po-2: Morgan Essex - *Evaluation of microbiome association models under realistic and confounded conditions*

Po-3: Vanessa Szott - *Potential efficacy of combined non-biosafety based intervention measures to reduce C. jejuni colonization in broiler chickens at slaughter age*

Po-4: Benjamin Reichelt - *Transmissions of Campylobacter spp. in the environment of commercial poultry farms in Germany*

Po-5: Anna Wessels - *Study concept: Impact of the intestinal microbiome on the regulation of appetite in pigs*

Po-6: Selina Schmidt* - *Effect of biocides on the evolution and spread of resistance* (Flash talk)

Po-7: Silver Anthony Wolf - *Computational insights into the gut resistome of horses receiving perioperative antibiotic prophylaxis revealed resistance gene accumulation throughout hospitalization*

Po-8: Laura Fuhrmann - *Effects of a specific pre- /probiotic combination and parent stock vaccination on performance and bacterial metabolites in broilers challenged with Escherichia coli*

Po-9: Marly Katerine Erazo Lugo - *Environmental microbes and Plasmodium transmission*

Po-10: Kimberly Hartl - *Stem cell competition in the context of injury-associated intestinal carcinogenesis*

Po-11: Joseph Nkamwesiga - *Peste des petits ruminants infection dynamics in Uganda*

Po-12: Robin Kempkens - *Impact of the microbiome and mucosal immune response on the production of pathogenic antibodies in patients with IgA nephropathy*

Po-13: Anika Sander - *Influence of intestinal hyperbilirubinemia on the gut microbiota and colonic inflammation*

Po-14: Lydia-Yasmin Sobisch - *Biocide resistance evolution in sulfate-reducing bacteria mediating microbially influenced corrosion*

Po-15: Friederike Gutmann - *Confounding matters in medical systems biology research*

Po-16: Katharina Zeilinger - *Ex-vivo screening assay of pre-and probiotic combinations for the inhibition of pathogenic Escherichia coli in piglets*

Po-17: Lisa Lemoine - *The skin microbiome `s influence on the toxicity of xenobiotics*

Po-18: Ahmed Abdelfattah - *Effects of soil microbiome on phyllosphere and aphid microbiome assembly*

Po-19: Daniel Höfle - *Metabolic capability of the vertically transmitted microbiome*

Po-20: Grace Klass - *Helminth-microbiota interactions in horses - How do cyathostomin infections affect the equine gut microbiome?*

Po-21: Pau De Yebra Rodo - *Aquatic Ecosystems: habitat and vector for Antimicrobial resistance (AMR) transmission*

Po-22: Syeda Anchel Zahra - *Effect of maternal overnutrition on melanocortin system development with a primary focus on gut brain axis*

Po-23: Ankur Midha* - *The microbiome of the intestinal parasitic nematode Ascaris suum is derived but distinct from its host* (Flash talk)

Po-24: Virginia Rossow - *OrkambiKIDS - Microbiome of CF-children*

Po-25: Hendrik Bartolomaeus* - *In-depth spatial analysis of microbiome-immune interaction in mice* (Flash talk)

Po-26: Rima Hayani - *Investigating the Microbiome of the Helminth Parasite Ascaris suum by Fluorescence in situ Hybridization*

Po-27: Tobias Goris* - *Transformation of flavonoids by human gut bacteria* (Flash talk)

Po-28: Aline Rosin - *Establishment of a commensal 3D-skin model for melanoma progression studies*

Po-29: Annika Oßwald - *Deoxycholic acid promotes colonic tumorigenesis in gnotobiotic mice colonized with a bile acid-converting simplified microbiota*

Po-30: Rebecca Luise Knoll* - *Patterns of gut microbial dysbiosis despite probiotic supplementation at day 28 of preterm life* (Flash talk)

Po-31: Jonathan Riedmüller - *Zinc supplementation in weaning piglets: A comparison of source and concentration*

Po-32: Carolina Schwedhelm - *Differences in the gut microbiome composition between individuals with or without obesity – Preliminary results from federated analyses in the context of the Knowledge Platform Intestinal Microbiomics (INTIMIC-KP)*

Po-33: Birgit Walther - *Perioperative antibiotic prophylaxis (PAP)-induced changes of the gut microbiota in horses elicit a common trajectory*

Po-34: Johannes Schulze Holthausen - *The effect of glutamine supplementation, birth weight and age on the dominant microbial composition in the stomach of suckling piglets*

Po-35: Jannike Krause - *Sample logistics affect structural and functional profiles of faecal microbiota* (Flash talk)

Po-36: Amelie Weber - *Microbial energy in immune homeostasis and function*

Po-37: Lisa Budzinski - *Multi-parameter ow cytometry identities distinct microbiota phenotypes in chronic inflammatory diseases*

Po-38: Aayushi Shah - *Understanding the Role of Microbiota Populations in Licensing of Pathogenic T cells in Intestinal Bowel Diseases*

Po-39: Anika Hartmann - Rheumatoid arthritis benefits from fasting and plant-based diet: an exploratory randomized controlled trial (NutriFast)

Po-40: Carola Ellner - *Effects of dietary rye and rapeseed on microbiota and electrophysiological parameters of the jejunum in weaner pigs*

Po-41: Isabel Dorst - *Beyond association and correlation: Data mining based on real world microbiome profiles*

Po-42: Noushin Arfatahery - *Identification of Vibrio kanaloa in the oyster Crassostrea gigas by fluorescent in situ hybridization*

Po-43: Sonja Wende* - *Revealing the patterns of bacteria and antimicrobial resistance from the gastrointestinal ecosystem in wild populations of house mice* (Flash talk)

Po-44: Víctor Hugo Jarquín Díaz - *Revealing the patterns of bacteria and antimicrobial resistance from the gastrointestinal ecosystem in wild populations of house mice*

Po-45: Lukasz Grześkowiak - *Influence of fiber composition in sows' diet on the colonization of gut pathogens in the offspring*

Anna Fagundes (Poster Po-1)

Charité Universitätsmedizin, Berlin

Respiratory tract microbiota modulates type 17 immunity and homeostasis of non-hematopoietic cells

While carrying out the vital function of gas exchange, the respiratory tract encounters a variety of microorganisms and environmental particles. Protection of the pulmonary microenvironment involves fine-tuned communication between the microbiota, immune and structural cells. The role of type 3 immune cells on integrating microbial cues into signals that reinforce lung barrier function has been well characterized during infection but remains poorly understood in homeostatic conditions. The respiratory microbiota is dynamic, and its density varies along the proximal-to-distal axis. Accordingly, the cellular composition of these regions is specialized to meet the local demands of mucociliary clearance and respiration. We hypothesize that type 3 innate immune cells contribute to the integration and coordination of these events in a spatio-temporal manner. To investigate the impact of the microflora in type 3 immunity and structural cells homeostasis, we characterized distinct regions of the respiratory tract and mice containing varied microbiota status. Our results demonstrate that microbial diversity and density directly correlate with a type 3 signature and enhanced activity of non-hematopoietic cells. We will further investigate what are the mechanisms underlying such processes and how their disruption might contribute to pathology.

Morgan Essex (Poster Po-2)

Experimental and Clinical Research Center (ECRC-MDC/Charité), Berlin

Evaluation of microbiome association models under realistic and confounded conditions

Testing for differential abundance is a crucial task in metagenome-wide association studies, complicated by technical or biological confounding and a lack of consensus regarding statistical methodology. Here, we developed a framework for benchmarking differential abundance testing methods based on implanting signals into real data. This strategy yields a ground truth for benchmarking while retaining the statistical characteristics of real metagenomic data, which we quantitatively validated in comparison to previous approaches. Our benchmark revealed dramatic issues with elevated false discovery rates or limited sensitivity for the majority of methods with the exception of limma, linear models and the Wilcoxon test. When additionally modeling confounders, we observed these issues to be exacerbated, but also that linear mixed-effect models or the blocked Wilcoxon test effectively address them. Exploratory analysis of cardiometabolic disease cohorts illustrates the confounding potential of medications and the need to consider confounders to prevent spurious associations in real-world applications.

The participant could not attend but agreed to include the work in this booklet

Vanessa Szott (Poster Po-3)

Freie Universität Berlin (FU), Berlin

Potential efficacy of combined non-biosafety based intervention measures to reduce *C. jejuni* colonization in broiler chickens at slaughter age

Campylobacteriosis was the most commonly reported foodborne gastrointestinal infection in the European Union (EU) in 2020. Therefore, reducing Campylobacter colonization in broilers at slaughter age is considered an important key step. Since previous measures are still insufficient on their own, we aimed to evaluate the efficacy of combined non-biosafety measures. Based on previous studies, we selected and combined four non-biosecurity-based interventions that were individually successful in reducing *C. jejuni* colonization. In fact, we combined a CE culture with bacteriophages and an essential oil (carvacrol) with organic acids. Per experiment, 58 newly hatched broiler chickens of breed Ross 308 were raised in the experimental animal facility on floor housing with litter. On day 10 of age, 12 broiler chickens (seeder) were orally inoculated with 104 cfu/500 µl *C. jejuni*. Thereafter, Campylobacter colonization and load was determined weekly by taking cloacal swabs of 23 randomly selected untreated broilers (sentinels). At the end of each experiment (33 days post hatch), sentinels were dissected and cecum and colon contents were collected for *C. jejuni* count determination. To examine the effect of a combination of carvacrol and organic acids, broilers were fed daily with 120 mg/kg feed of carvacrol and a mixture of four acids in their drinking water. Moreover, to evaluate the efficacy of the combination of a CE-culture with bacteriophages, broiler chickens were treated with the CE-culture twice (day 1 using spray application, day 25 via drinking water application) and received a phage combination of two phages continuously via drinking water four, three, and two days prior to necropsy. Broilers were provided free access to commercial broiler feed and filtered water from the municipal water supply ad libitum during the entire study. Cecal count enumeration demonstrated that the *C. jejuni* load was significantly reduced for the group receiving a combination of the CE-culture and bacteriophages compared with the control group (log reduction of 1.0 log10 MPN/g). Likewise, colon counts were significantly decreased for the group receiving a combination of bacteriophages and the CE-culture (log reduction of 1.0 log10 MPN/g) In contrast, although we observed a log reduction of 1.0 log10 MPN/g in C. jejuni cecal counts in the group receiving a combination of carvacrol and organic acids, this reduction was, however, nonsignificant compared with the control group. Likewise, there was no significant difference in *C. jejuni* counts in the colon. We conclude that a combination of this particular CE-culture and bacteriophages may be a promising practical approach in broiler production to reduce *C. jejuni* colonization in broiler chickens at slaughter age. However, why the combination of carvacrol and organic acids failed to reduce *C. jejuni* intestinal colonization is unclear and remains to be investigated.

Benjamin Reichelt (Poster Po-4)

Freie Universität Berlin (FU), Berlin

Transmissions of *Campylobacter* spp. in the environment of commercial poultry farms in Germany

Campylobacter (C.) jejuni is the most common cause of campylobacteriosis in humans, and broiler meat is considered one of the major sources. In general, it is believed that the poultry house environment may be a reservoir for *Campylobacter* spp. to some extent. However, to date, there are limited data on the spillover of *Campylobacter* from houses with *Campylobacter*-positive flocks and the buildup of environmental reservoirs. In addition, *Campylobacter* spp. are capable to transit into a viable but non-culturable (VBNC) state as a result of various extrinsic stress factors. In order to identify possible reservoirs of persistent and VBNC-Campylobacter and thus uncover relevant transmission pathways, a longitudinal study was conducted. Material/Methods: Broiler farms and their environment close to the barn were intensively investigated at the end of two consecutive fattening cycles in summer and winter. In order to draw careful conclusions about possible transmission between consecutive fattening cycles, the selected farms were also examined after cleaning and disinfection. All samples were processed according to the semi-quantitative method for the detection and enumeration of *Campylobacter* spp. (ISO/TS 10272-3). Selected isolates were species-typed by MALDI-ToF analyses. A systematic selection of isolates from all sampling collections was examined by whole genome analyses. Moreover, environmental and selected broiler house samples were treated simultaneously with propidium monoazide (PMA) and analyzed by live/dead discrimination using real-time PCR (qPCR) in the further course of the study Results: In two out of three farms, *Campylobacter* was frequently detected in high amounts in the chicken barns, especially in summer. However, the pathogen was only occasionally detectable in the environment, particularly in water-associated matrices, especially in winter. However, *Campylobacter* could not be isolated in broiler houses after cleaning and disinfection. The emission source of culturable Campylobacter was found to be primarily contaminated chicken manure. C. jejuni proved to be the dominant species of the isolates examined. PMA-qPCR revealed no detection of VBNC-Campylobacter in selected barn and environmental samples. In contrast, *Campylobacter* DNA was frequently detected in environmental samples. The present study provides insight into the significance of *Campylobacter* in the environment in relation to prevalence in the broiler farms investigated in Germany. The results established indicate sporadic environmental findings in the immediate vicinity, suggesting spread, persistence and possible reintroduction. *C. jejuni* was found in nearby water bodies, indicating that the pathogen is ubiquitous by spread and circulation. Although the findings were sporadic and no significant source of transmission has yet been identified, it should be kept in mind that even very low levels of *Campylobacter* may colonize whole poultry flocks.

Anna Wessels (Poster Po-5)

Freie Universität Berlin (FU), Berlin

Study concept: Impact of the intestinal microbiome on the regulation of appetite in pigs

Beneficial interplay between host and microbiome is critical for maintaining host physiological and immunological functions, while disease is often associated with microbial dysbiosis. Differences in gut microbial communities have been identified in pigs with feed intake-related differences in feeding efficiency. Numerous commensal and pathogenic bacteria synthesize peptides in the gastrointestinal tract that resemble host "satiety" peptides and may influence central appetite regulation by acting on the appropriate neurons in the hypothalamus. The diversity of microbial metabolites appears to serve primarily for microbial exchange and as substrates for host intestinal cells. However, microbial metabolites also stimulate satiety hormones and neurotransmitters, potentially influencing host behavior related to feed intake. Based on these findings, a mechanistic relationship between the gut microbiome and host eating behavior is hypothesized, which affects meal quantity and quality. The causal chain is as follows: The nutrient base determines the microbial pattern of the digestive tract. After consolidation, the microbiome of the main fermenting organ influences feed selection by affecting the physiology of the host to obtain the nutrients to which it is adapted. Accordingly, a study is planned to address the following hypotheses: i) microbial metabolites contribute to the modulation of feeding behavior by stimulating peripheral and central satiety mediators, as well as reward systems in the brain, and ii) the composition of the colon microbiota affects host taste preferences by influencing the expression of taste receptors in the oral cavity via metabolites, thus favoring the intake of preferred food components. To this end a study with pigs is planned as follows: The microbiome of 48 pigs will be consolidated over 4 weeks using the dietary set points protein and fiber. The extent to which the microbial community and metabolites have changed in the pigs will then be assessed. The concentrations of satiety hormones and neurotransmitters, the expression of satiety mediators in the brain and taste receptors in the oral cavity in relation to the microbial composition will be compared. In addition, a subgroup will be observed in a choice experiment with respect to their food preferences to test whether the pigs stick to their experimental diet or prefer a different one. The results of the project should provide information on whether the gut microbiome can influence the host with regard to its food choice.

Selina Schmidt (Poster Po-6 / Flash talk-1)

Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin

Effect of biocides on the evolution and spread of resistance

Biocides used as material preservatives are in contact with various environments during direct application or passive leaching from protected materials [1]. Antimicrobial resistance (AMR) is a global health problem and the environment is an important compartment for the evolution and transmission of AMR [2,3]. Soil is an environment with a large reservoir of natural microbial communities and antimicrobial resistance (AMR) genes. Those natural microbial communities are frequently exposed to biocides used as material preservatives. Previous studies have shown that antibiotics, metals and pesticides affect the underlying processes of resistance evolution and spread; namely de novo mutagenesis and horizontal gene transfer by conjugation and transformation in microbial communities. However, it is unknown if active substances used in material preservatives are involved in these processes. We show that biocides used as material preservatives affect rates of mutation and conjugation in microorganism in a species- and substance-dependent manner, while rates of transformation are not directly affected. Our data highlights the importance of assessing the contribution of material preservatives on AMR evolution and spread in the environment.

Silver Anthony Wolf (Poster Po-7)

Robert Koch Institut (RKI), Berlin

Computational insights into the gut resistome of horses receiving perioperative antibiotic prophylaxis revealed resistance gene accumulation throughout hospitalization

Antimicrobial resistance (AMR) is an emerging global One Health issue, affecting human and veterinary medicine, as well as the environment. Previous research revealed that clinics providing health care for companion animals, such as horses, are indeed “hot spots” in respect to the local spread of multidrug resistant (MDR) bacteria. Horses receiving gentamicin/penicillin (GP) as a perioperative antibiotic prophylaxis (PAP) were frequently colonized with MDR pathogens. Since the duration of antibiotic therapy influences the local selective pressure and recovery time of the enteral microbiome, two distinct GP-PAP regimens were comparatively investigated, particularly in regards to the emergence of genes conferring resistance to antibiotics (ARGs). Hospitalized horses subjected to colic surgery received GP-PAP, either as a single dosage (SSG) regimen or across 5 consecutive days (5DG). Fecal samples were collected on day 0 (hospital admission), 3 and 10 (post-surgery). In total, sample sets of 12 horses (n = 36) were metagenome shotgun sequenced and computationally analysed. The resistome was characterized and tested for statistical correlation with the study group and diversity indices. The results display unique gut metagenomes associated with each of the equine patients. Beyond the impact of hospital stay and surgery, GP-PAP caused microbiome perturbations, resulting in an average decrease of the α-diversity on day 3 (5DG = -2.06, SSG = -1.66) followed by a recovery process at day 10 (5DG = +2.09, SSG = +0.99). Resistome analysis revealed a strong increase in normalized ARG abundance, particularly for the 5DG, at day 3 (5DG = +4.6 fold change (FC), SSG = +3.6 FC) and subsequent decrease on day 10 (5DG = -6.8 FC, SSG = -1.3 FC). ARG accumulation was found to be statistically significant for day 3 of the 5DG ($p = 0.032$). Furthermore, ARG abundance and α-diversity were found to be negatively correlated (spearman, $r = -0.62$, $p = 8.2e\text{-}05$) across the sample set. Our preliminary results help to establish an understanding of the multifaceted effects of hospitalization and antibiotic prophylaxis on the equine gut microbiome. Whereas the decrease in α-diversity was expected, we also describe a strong increase in ARGs, especially within the 5DG. This is in line with the observed increase of MDR bacteria colonizing the equine patients. While the beneficial effect of decreased selective pressure caused by the SSG treatment is plausible, additional analyses are required to further describe the influence of the different PAP regimens on the microbiome, particularly in regards to MDR bacteria.

The participant could not attend but agreed to include the work in this booklet

Laura Fuhrmann (Poster Po-8)

Freie Universität Berlin (FU), Berlin

Effects of a specific pre-/probiotic combination and parent stock vaccination on performance and bacterial metabolites in broilers challenged with *Escherichia coli*

Antibiotic resistance poses a risk for human and animal health, increasing the demand for effective alternatives. Thus, we aimed to investigate if a strategy, combining nutritional tools and parent-stock-vaccination, enhances performance and bacterial metabolism in broilers challenged with an antibiotic resistant *Escherichia coli*. Methods: 225 one-day-old Ross 308 chicks (male and female) were randomly assigned to five treatment groups with nine birds per pen and five replicates. The groups negative control (Cn), positive control (Cp) and parent-stock-vaccination (VAC) received a diet based on corn, wheat and soybean meal. Two groups (FA and FA-VAC) received the same diet supplemented with the probiotic *Enterococcus faecium* and prebiotic oligofructose (FOS). Groups VAC and FA-VAC derived from vaccinated hens (E. coli O1/O18, inactivated). At day eight of life, broilers in groups Cp, FA, VAC, and FA-VAC were orally challenged with a multidrug resistent *E. coli* O1/O18 field isolate. Bodyweight and feed intake were obtained weekly. After four weeks, two chickens per pen were sacrificed. Crop and caecal contents were analysed for pH and bacterial metabolites. Data were subjected to ANOVA and post hoc Tukey-HSD (performance, pH) or Kruskal-Wallis test with Bonferroni correction (metabolites). Differences were considered significant at $p \leq 0.05$. Results: FA-VAC-birds achieved higher body weights compared to controls throughout the experiment. FA and FA-VAC showed lower caecal pH-levels and higher crop L-lactate concentrations than controls. In FA-VAC-birds, there was also a trend toward decreased pH in the crop. Caecal total fatty acids, acetate and n-butyrate concentrations increased in VAC and FA-VAC-animals compared to Cn. Conclusion: *Enterococcus faecium* and FOS combined with parent-stock-vaccination improved body weights in *E. coli* challenged broilers. Additionally, pH-levels decreased and bacterial metabolites increased in caecum and crop digesta of broilers receiving both preventive treatments.

Marly Katerine Erazo Lugo (Poster Po-9)

Max Planck Institute for Infection Biology (MPIIB), Berlin

Environmental microbes and *Plasmodium* transmission

Malaria remains one of the deadliest diseases in the world. It is caused by *Plasmodium* parasites that are transmitted to humans through the bite of infected female mosquitoes of the genus Anopheles. The transmission of *Plasmodium* parasites is strongly influenced by environmental factors that shape the dynamics of mosquito populations. Among these environmental factors are the microbial communities that inhabit the mosquito midgut. Commensal bacteria, in particular, have been shown to be essential for normal larval development and are key players in modulating such physiological functions as nutrient acquisition and immune response. In fact, previous studies in the laboratory have shown that commensal bacteria can influence the development of human malaria *P. falciparum* parasites. However, the identity and contribution of specific microbial species or communities to the mosquito - *Plasmodium* interactions remain unknown. In the first part of the project, I established the methods to generate and characterize female *Anopheles coluzzii* associated with a defined midgut microbiome. To this end, after microbiota elimination with antibiotics, single bacterial species or microbial communities were fed to mosquitoes with sugar for gut recolonization. The generation of mosquitoes associated with defined mosquito microbial multispecies consortia with high controllability will later be used to determine metabolic relationships between individual and mixed bacterial species on development of *P. falciparum* and transmission. The findings obtained during this project should enhance our understanding of the environmental factors that modulate malaria transmission and could offer new insights for vector control strategies.

The participant could not attend but agreed to include the work in this booklet

Kimberly Hartl (Poster Po-10)

Charité, Berlin

Stem cell competition in the context of injury-associated intestinal carcinogenesis

Colorectal cancers arise by a defined sequence of mutations that differs between sporadic and inflammation-associated (IA) cancers. In IA tumors, mutations that cause a loss of the tumorsupressor P53 occur earlier. P53 mutant cells were able to outcompete P53 wild type (wt) cells in a mouse model of colitis (Vermeulen et al.) but mechanistic insights are lacking. Therefore, we have developed murine and organoid-based models to study the context dependent role of Trp53 knock-out. Trp53 KO organoids did not exhibit phenotypic difference compared to wt organoids in full medium. Similarly, loss of Trp53 did not cause severe perturbations of crypt architecture in vivo. In contrast, when we mimic epithelial injury in vitro, we observe a selective advantage of Trp53 KO cells through activation of regenerative pathways. Similarly, in a mouse model of colitis, regenerative Trp53 KO cells expand and persist after the primary epithelial insult. Additionally, treatment with microbial metabolites with which stem cells could come into contact upon tissue damage, reveals differences between Trp53 KO and wt organoids in growth behavior. Further in vitro and in vivo experiments will explain how the damaged epithelium selects for P53 mutant cells, which might help to develop screening and preventive measures for patients.

The participant could not attend but agreed to include the work in this booklet

Joseph Nkamwesiga (Poster Po-11)

Freie Universität Berlin (FU), Berlin

Peste des petits ruminants infection dynamics in Uganda

Peste des Petits Ruminants (PPR) is a transboundary, highly contagious, and fatal disease of small ruminants. PPR causes global annual economic losses of between USD 1.5-2.0 billion across more than 70 affected countries. Despite the commercial availability of effective PPR vaccines, lack of financial and technical commitment to PPR control coupled with a dearth of refined PPR risk profiling data in different endemic countries has perpetuated PPR virus transmission. In Uganda, over the past five years, PPR has extended from north-eastern Uganda (Karamoja) with sporadic incursions in other districts /regions. To identify disease cluster hotspot trends that would facilitate the design and implementation of PPR risk-based control methods (including vaccination), we employed the space-time cube approach to identify trends in the clustering of outbreaks in neighbouring space-time cells. We also used negative binomial and logistic regression models and identified high small ruminant density, extended road length, low annual precipitation and high soil water index as the most important drivers of PPR in Uganda. The study identified (with 90 - 99% confidence) five PPR disease hotspot trend categories across subregions of Uganda. Diminishing hotspots were identified in the Karamoja region whereas consecutive, sporadic, new, and emerging hotspots were identified in central and southwestern districts of Uganda. Inter-district and cross-border small ruminant movement facilitated by longer road stretches and animal comingling precipitate PPR outbreaks as well as PPR virus spread from its initial Karamoja focus to the central and south-western Uganda. There is therefore urgent need to prioritize considerable vaccination coverage to obtain the required herd immunity among small ruminants in the new hotspot areas to block transmission to further emerging hotspots. Findings of this study provide a basis for more robust timing and prioritization of control measures including vaccination.

Robin Kempkens (Poster Po-12)

German Rheumatism Research Centre (DRFZ), Berlin

Impact of the microbiome and mucosal immune response on the production of pathogenic antibodies in patients with IgA nephropathy

IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide. Clinical courses range from benign courses with proteinuria and arterial hypertension to progressive courses with chronic renal failure. The pathophysiology is often summarized with a 4-hit hypothesis: (1) An increased expression of galactose-deficient IgA1 (gd-IgA1) and (2) the formation of autoantibodies directed against the hinge region of gd-IgA1 resulting in (3) the formation of immune complexes. Their glomerular deposition leads to (4) fibrosis and chronic inflammation. What triggers the formation of the pathogenic antibodies and where they are formed is still enigmatic. It has been hypothesized that the microbiome is involved in the etiopathogenesis of IgAN. In this project, I propose to (1) elucidate whether and how the microbiota modulates B and plasma cells leading to the production of gd-IgA1 antibodies, and (2) to investigate the role of the microbiota in the induction of the pathogenic autoantibodies. The microbiome will be analyzed using microbiota flow cytometry and different experimental setups will help to establish a causal relationship to the triggered pathogenic immune response. Thus, the project will close a crucial gap in the pathophysiological understanding of IgAN, which is an important step to develop targeted therapies to specifically treat progressive clinical courses.

Anika Sander (Poster Po-13)

Deutsches Institut für Ernährungsforschung (DIfE), Potsdam

Influence of intestinal hyperbilirubinemia on the gut microbiota and colonic inflammation

Gilbert's Syndrome (GS) is a benign condition caused by a gene polymorphism of the UGT1A1 gene, that reduces the activity of the uridine diphosphate glucuronosyltransferase (UGT) 1A1 and is clinically characterized by elevated levels of bilirubin in the serum. Epidemiological studies demonstrated an inverse correlation of GS and inflammatory bowel diseases (IBD). We hypothesized that this may be mediated by altered bilirubin levels influencing the microbiota and the metabolism in the intestine, by acting as ligand of the aryl hydrocarbon receptor (AhR) in the intestinal epithelium. Two rodent models of serum hyperbilirubinemia were used: Gunn rats that have an inherited hyperbilirubinemia due to a loss of hepatic Ugt1a1, and C57BL/6 wildtype mice with induced hyperbilirubinemia. Colonic microbiota was analyzed by 16S rRNA gene sequencing analysis, epithelial bilirubin was measured using LC-MS/MS and in the serum, 3-nitrotyrosine (3-NT), an inducible NO synthase (iNOS)-derived reactive nitrogen species, was measured by HPLC. Gene expression was measured by qPCR and colonic tissue was analyzed using immunofluorescence staining. Bilirubin levels in the colonic mucosa were higher in Gunn compared to control rats, indicating 'intestinal hyperbilirubinemia' conditions. Gunn rats demonstrated a distinct shift in gut microbial composition, resulting in higher numbers of Gram- positive bacteria and lower levels of Gram-negative bacteria. Surprisingly, Akkermansia muciniphila was exclusively present in Gunn but not in the control rat colonic microbiota. As bilirubin functions as an agonist of AhR, Ahr expression was elevated in the colonic epithelium of Gunn rats, whereas the gene expression of downstream targets Inf-γ and Nos2 was reduced. This was confirmed by lower levels of iNOS detected in the colonic mucosa. To confirm changes observed in the rat model, we performed intraperitoneal bilirubin injections in wildtype mice for 10 consecutive days to mimic hyperbilirubinemia. Being part of an ongoing analysis, mice in the bilirubin group (n=10) had lower bodyweight than the control group (n=9) and the small intestine and caecum of these mice were significantly lighter and shorter than the control group. Short-term DSS treatment of Gunn and control rats led to shorter colon lengths in the control group (n=6), indicating that Gunn rats were less affected by DSS-induced colitis. Lower levels of 3-NT in plasma of Gunn rats confirmed reduced iNOS activity. The Gunn colonic microbiota showed no distinct clustering with or without DSS-induced colitis, suggesting a weaker effect strength by DSS treatment under intestinal hyperbilirubinemia conditions. In contrast, the control rat microbiota showed distinct compositional spreading according to DSS treatment, suggesting that the colonic microbiota was more affected by DSS- induced colitis. Bilirubin regulates the microbiota composition in the colon and may have beneficial effects for the homeostasis of the microbiota, potentially reducing the risk of colonic inflammation.

Lydia-Yasmin Sobisch (Poster Po-14)

Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin

Biocide resistance evolution in sulfate-reducing bacteria mediating microbially influenced corrosion

Sulfate-reducing bacteria (SRB) are environmentally and industrially important microorganisms. In addition, SRB play a key role in the gut microbiome. The disadvantage of their metabolic activity, such as sulfate reduction, results in the formation of toxic sulfide that leads to microbial Influenced corrosion (MIC). SRB have been responsible for biocorrosion of ferrous metal structures in different industries e.g. petroleum and paper industry, and waste water production. One of these control measures is the use of biocides. However, it has been shown that various bacteria develop antimicrobial resistance due to excessive use of biocides. Thus, a deeper understanding of the evolutionary trajectories towards biocide resistance of SRB is necessary. We apply three commonly used biocides, tetrakishydroxymethyl phosphonium sulphate (THPS), benzalkonium chloride (BAC), and glutaraldehyde (GLUT) to investigate the susceptibility of two SRB that are known to cause MIC, namely Desulfovibrio alaskensis G20 (G20) and Desulfovibrio vulgaris Hildenborough (DVH). We determined the minimum inhibitory and bactericidal concentration, and time-kill kinetics of THPS, BAC, and GLUT. The susceptibility data is used to conduct ongoing evolution experiments to determine the evolution of resistance towards biocides of SRBs. For both SRB strains, a genome-wide, barcode-tagged transposon mutant library is available. Both transposon mutant libraries have been exposed to sub-inhibitory biocide concentrations, and the abundance of mutants will be detected via Tn-Seq. The combined data from the evolution and the transposon mutant library experiments will provide to a deeper understanding of the genes involved in biocide resistance. The outcome of this work will shed light on the basic stress response of SRB and improve the management of MIC.

Friederike Gutmann (Poster Po-15)

Experimental and Clinical Research Center (ECRC-MDC/Charité), Berlin

Confounding matters in medical systems biology research

An erroneous study design can often have detrimental effects on a scientific study, wasting financial resources and, in the worst case, needlessly harming animals and human lives. One example of such an inaccurate research scheme became apparent in a project aiming to characterize the perioperative metabolome in colorectal (CR) surgery. Here, we could show that postoperative metabolomic profiles vary between cancer and benign disease patients. However, since both disease entities are treated differently surgically, it is impossible to trace these effects back to either the surgery or the disease. Yet another threat to the success of a research project are variables that influence independent and dependent variables simultaneously and thus impede disentangling true effects. In a project aiming to find associations of microbial features with atrial fibrillation (AF), for example, we found the association between AF and the Barnesiella and Collinsella genera to be not confounded by any of the study's metavariables, including age, medication, and dietary patterns, but co-associated with aspirin intake. This suggests a concurrent cardioprotective role of the microbiome and aspirin intake. These results were calculated using an in-house developed pipeline that applies multivariate regression analysis to determine the driver of an association. Especially lasting, highly diverse diseases accompanied by varying comorbidities naturally come with a large pool of potential confounders. One example is multiple sclerosis (MS), which is at the center of one of the presented projects. In this project, we aim to investigate the microbiome-metabolome-brain axis and the role of dietary interventions in treating autoimmune diseases. We will analyse the gut microbial composition and conduct a metabolomics analysis of stool and plasma samples of MS patients consuming different diets. As deducible from the projects described above, the following data analysis needs to include careful control for confounding to avoid untraceable and exclude false-positive results but also reveal other drivers of disease development by accounting for the numerous study variables potentially influencing both multiple sclerosis progression and the microbiome. Taken together, I am presenting three different projects, each influenced by confounding on different levels and to varying extents, but all emphasizing the necessity to consider confounding effects when developing statistical analysis workflows for systems medicine research.

Katharina Zeilinger (Poster Po-16)

Institut für Tierernährung, Freie Universität Berlin (FU), Berlin

Ex-vivo screening assay of pre-and probiotic combinations for the inhibition of pathogenic *Escherichia coli* in piglets

Introduction: Although pro-/prebiotics have been widely used in pig production, there are still inconsistent results on their efficacy. Beneficial effects depend not only on the probiotic strains or the prebiotic structure, but also on dose, animals and farm management. Pro-/prebiotics should be applied in a targeted way, including farm individual conditions. The aim of this study was to develop an ex-vivo screening assay to find tailor-made combinations of pro- and prebiotics for individual farms for the inhibition of enteropathogenic *Escherichia coli* (EPEC). Animals, material and methods: Fecal samples were obtained from 20 German pig farms. Three samples from each farm were used for ex-vivo analyses. Three probiotics (*Bacillus licheniformis* and *Bacillus subtilis, Saccharomyces cerevisiae boulardii, Enterococcus faecium*) and three prebiotics (Inulin, Fructooligosaccharide, Mannan-oligosaccharide) were selected alone or in combination to test their effectiveness to inhibit the growth of an EPEC model strain in fecal samples. Fecal slurries, mixed with pro-/prebiotics and the EPEC strain were incubated anaerobically for 24h. Aliquots of the incubated slurries were transferred to an antibiotic containing medium. Growth was recorded (OD690nm) for 24 h in a microplate reader. Comparison of exponential growth phase OD values were used to analyze the survival of the EPEC strain. A mixture of three antibiotics (cloxacilline, metronidazole, vancomycin) was used to ensure selective growth of the EPEC model strain. The specificity was confirmed by the absence of growth in all fecal samples not inoculated with the EPEC. Results and discussion: Analysis of the growth curves showed that the time point of 8 h was most suitable to compare EPEC growth in controls to pre-/probiotic supplemented samples. Suppression of *E. coli* growth was not always observed, as some pro-/prebiotic combinations also led to increased growth of the model strain. Our results indicate that the survival of the model strain depended not only on the added pre/probiotics, but also on the origin of the fecal samples used. Thus, in-farm variation of *E. coli* growth was much lower than differences between farms. The ex-vivo assay depends on a suitable antibiotic mixture to allow specific growth of a pathogen model strain after incubation with pre-/probiotics. The use of fecal samples includes the response of the individual microbiota and is therefore preferred to in-vitro assays. The developed method has the potential to find tailor-made combinations of pro- and prebiotics for individual farms to inhibit pathogenic E. coli, as many combinations can be tested in parallel.

Lisa Lemoine (Poster Po-17)

Bundesinstitut für Risikobewertung (BfR), Berlin

The skin microbiome`s influence on the toxicity of xenobiotics

Exposure to xenobiotic pollutants and substances has repeatedly been associated with adverse health effects. While the majority of reported cases still relate to direct substance effects, there is increasing evidence that microbiome-dependent metabolism of xenobiotic substances can also have adverse effects on the host. This may be due to microbial biotransformation of compounds, interaction between the microbiota and endogenous host detoxification enzymes, or altered bioavailability of xenobiotics. Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (B[a]P) are among the most abundant environmental pollutants and contaminants. However, the effects of the skin microbiota on the uptake, metabolism and distribution of B[a]P in humans remain unclear. In our previous work, we demonstrated that skin isolates are capable of degrading benzo[a]pyrene (B[a]P) in batch cultures, forming both cytotoxic and genotoxic metabolites, some of which are distinct from eukaryotic ones. Building on these results, in this work we investigated the corresponding microbial B[a]P metabolism and toxification as well as potentially relevant microbiome-host interactions using a recently developed microbially competent 3D-skin model. Using GC/MS analysis, we were able to show that the skin microbiota metabolizes B[a]P on/in human skin. However, notably, unmetabolized B[a]P as well as its metabolites penetrated the skin layers of commensal colonized models to a lesser extent due to strengthening of tight junctions and epidermal differentiation. Most importantly, the formation and penetration of the ultimate carcinogen BPDE was greatly reduced, resulting in lower BPDE-DNA adduct formation.The results show significant implications of the skin microbiome for the toxicology of external chemical exposures. The newly founded junior research group “Skin Microbiome” of the BfR will investigate the role of both the skin and the gut microbiome in substance-associated acute and chronic toxicity. This also includes the potential influence of the skin microbiome on tumorigenesis of skin carcinomas. For this purpose, we are expanding our microbially colonizable 3D skin models, which are not available outside the BfR and which can be used to investigate fundamental toxicological interactions, and transferring this method to melanoma models. Substances that prove to be of interest will then also be studied in the environment of the gut microbiome in the coming years.

Ahmed Abdelfattah (Poster Po-18)

Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam

Effects of soil microbiome on phyllosphere and aphid microbiome assembly

All multicellular organisms are now known to be associated with diverse microbial communities. Identifying the mechanisms by which these communities are assembled is crucial to understanding community dynamics. Here we aimed at identifying the effect of manipulating soil microbiome on the assembly of oak phyllosphere and aphid's microbiomes. We further evaluated the impact of aphid herbivory on the phyllosphere and soil microbiome. We used microcosms, which physically separate above- and below-ground compartments, to grow oak seedlings in three soils with different microbiomes yet the same physiochemical properties. Amplicon sequencing and RT-qPCR were used to characterize and quantify the bacterial and fungal communities in soils, leaves, and aphids. Soil microbial community composition had a significant effect on the fungal and bacterial community compositions of leaf and aphid microbiomes, indicating assembly processes, at least partially, from soil to aphids through plants. Aphid herbivory significantly decreases microbial alpha diversity in leaves. Leaf fungal community composition and soil bacterial community composition shifted upon aphid herbivory.

Daniel Höfle (Poster Po-19)

Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam

Metabolic capability of the vertically transmitted microbiome

Plant endosphere is a specific habitat for endophytic microbial communities where essential functional interactions occurs. Recently, it has been shown that plants transmit distinct microbial communities via seeds to their offspring. Regarding the metabolic capability of these microorganisms information is lacking, although metabolic functions of the microbiome are a major factor in plants as they greatly increase their metabolic repertoire; it can improve plant growth, health and resistance to biotic and abiotic stress. The aim of this study was to investigate the metabolic capability of the transmitted bacteria in apple plants and the differences in microbial functional diversity between phyllosphere and roots. Several techniques were used including Biolog EcoPlates for metabolic analysis, Illumina MiSeq V3 for identification of the vertically transmitted bacteria, qPCR and bioinformatics to analyse the transmitted seed microbiome. The study showed that species richness, Shannon diversity as well as abundance of bacteria were higher in roots than phyllosphere. Further, the metabolic capacity was different; main differences of carbon metabolization occurred in D-xylose, L-phenylalanine, 4 hydroxybenzoic acid, α-cyclodextrin and glycogen. Carbon source had a significant impact on species richness and Shannon diversity in roots, but not in phyllosphere. In general, the composition of the bacterial rhizosphere microbiome was more diverse than the microbiome of the phyllosphere and both were dominated by *Pseudomonas* (phyllosphere: 88.2 %, roots: 84.4 %) and *Yersinia* (phyllosphere: 11.7 %, roots: 15.5 %).

Grace Klass (Poster Po-20)

Freie Universität Berlin (FU), Berlin

Helminth-microbiota interactions in horses - How do cyathostomin infections affect the equine gut microbiome?

Cyathostomins are the most common endoparasites in equines worldwide and currently comprise of 50 recognized species. While they can cause severe illness and death in some, virtually all horses with access to grazing pasture are constantly (re)infected with cyathostomins. Why, when and how some of these infections progress to clinically relevant cases is still unknown. While infections with Anoplocephala spp. are considered to be less prevalent, even minor worm burdens can cause severe colic in immunocompromised horses, e.g. foals, youngstock or pregnant mares. Recent studies have underlined the relevance of the intestinal microbiome to the equine immune system and suggested links to nematode infections and other diseases, although the underlying pathomechanisms are yet to be elucidated. Furthermore, anthelmintic resistance is an increasingly emergent issue in Cyathostomins, threatening adequate treatment of serious infections. Current prevalence data for Anoplocephala in foals and mares in Germany is lacking. Little to nothing is known about the prevalence, pathogenicity and anthelmintic resistance of individual Cyathostomin species, which is largely due to difficulties in morphological species differentiation. Advances in PCR and DNA sequencing have significantly improved cyathostomin species differentiation methodology and provided basic data essential to clarifying their phylogeny and studying anthelmintic resistance. Furthermore, next generation sequencing approaches can be employed to monitor the equine intestinal microbiome and nemabiome. These data can subsequently be used to study interdependencies of cyathostomins and Anoplocephala with the microbiome. During this project, a longitudinal study will be conducted on three warmblood studs in Germany. It aims to provide data regarding (i) prevalence of individual Cyathostomin species and potential life-time associated changes in cyathostomin species composition, (ii) prevalence of Anoplocephala spp. in certain age groups, (iii) development of the intestinal microbiome in foals and their dams over time. Subsequent analysis will study connections between (v) the species composition of cyathostomin populations and the intestinal microbiome composition in their host, (vi) Anopolocephala infections and the intestinal microbiome composition.

Pau De Yebra Rodo (Poster Po-21)

Leibniz Institute for Zoo and Wildlife research (IZW), Berlin

Leibniz Institute for Freshwater Ecology and Inland Fisheries (IGB), Berlin

Aquatic Ecosystems: habitat and vector for Antimicrobial resistance (AMR) transmission

Antimicrobial resistance (AMR) has been declared by the World Health Organization (WHO) as one of the top 10 global public health threats in the presence and near future. In a recent study, data from 204 countries suggest a burden of 1,27 million deaths associated with AMR bacteria in 2019 [1]. One of the challenges of monitoring AMR organisms and antimicrobial resistance genes (ARGs) is their ubiquitous distribution, from living organisms to natural environments like water, soil and air. In particular, water plays a key role as a vector and reservoir for AMR transmission across urban and rural areas [2]. Our preliminary results suggest that the abundance of AMR in urban waters and sediments is substantially higher compared to rural water bodies. In addition, rural lakes sediments and water derived from farmlands is observed to be a major source of environmental AMR due to frequent antibiotics use for cattle raising. My research project seeks to provide further understanding on AMR distribution in aquatic ecosystems as well as to characterize the humanization of urban water microbiomes. In particular, the use of long-read sequencing in this project will allow to recover mobile genetic elements (MGEs) such as plasmids and genomic islands (GIs) [3] and thus a higher resolution in the metagenome assembly. The results from this project will allow to directly link frequency and diversity of AMR and the carrying bacteria, providing a broad overview of AMR profiles in different waters and potential disease vectors of concern in the area of Berlin-Brandenburg. [1] Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022 Jan 18:S0140-6736(21)02724-0. doi: 10.1016/S0140-6736(21)02724-0. Epub ahead of print. PMID: 35065702. [2] Karkman, A., Pärnänen, K., & Larsson, D. J. (2019). Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nature communications, 10(1), 1-8. [3] Maguire, F., Jia, B., Gray, K. L., Lau, W. Y. V., Beiko, R. G., & Brinkman, F. S. (2020). Metagenome-assembled genome binning methods with short reads disproportionately fail for plasmids and genomic islands. Microbial genomics, 6(10).

Syeda Anchel Zahra (Poster Po-22)

Effect of maternal overnutrition on melanocortin system development with a primary focus on gut brain axis

The prevalence of obesity across the globe is rising at an alarming rate. Research in recent years has demonstrated that early life exposure to adverse maternal nutritional environments impairs the hypothalamic neurocircuit development, which is involved in regulating energy homeostasis during adulthood and beyond. Maternal overnutrition leads to hypothalamic AgRP neurons which result in impaired insulin and leptin responsiveness. Interestingly, recent studies uncovered a role of endoplasmic reticulum stress in the onset of changes to neuronal connection in the developing brain, suggesting that reducing aspects of ER stress during pregnancy can have beneficial effects on brain development and lifelong metabolism of the offspring. Therefore, it is imperative to understand the critical developmental periods during which this inflammation can be targeted and lifelong alterations in the hypothalamic neural circuitry attenuated. Recently a link between brain development and ER stress has been implicated with potential links specifically to the peripherally derived bile acids which are modulated by the microbiome. Intestinal bacteria regulate the composition of the brain bile acid pool which plausibly acts as a communication medium between the brain and the gut microbiome. However, the precise mechanism by which bile acids communicate within the gut-brain axis is yet unknown. Research has implicated the neuroprotective and anti-inflammatory potential of tauroursodeoxycholic acid (TUDCA), which is a bile acid conjugate, in several animal models of neurological disorders. As bile acid signaling to the brain occurs through both direct and indirect pathways plausibly mediated through central FXR and TGR5 signaling and glucagon like peptide-1 (GLP-1) and fibroblast growth factor 19 (FGF19) respectively. I will therefore explore the effect of maternal metabolic state, i.e. overnutrition, on circulating levels of gut derived metabolites, such as TUDCA, and to determine if an alteration in TUDCA due to changes in the microbiome is causative of neurodevelopmental changes. To achieve my objectives, I will use both a traditionally housed mouse model and a gnotobiotic mouse model of maternal overnutrition. Both models will be colonized with a defined human microbial consortium that I will verify to be capable of inducing altered levels of a precursor to TUDCA in the circulation. The effects of the modulated TUDCA levels and the altered bile acid pool will then be tested through biochemical, histopathological, and immunohistochemical assays. The results of the current project will give important insights into the molecular cross-talk between microbiome gut brain axis modulated by TUDCA through ER stress mitigating potential.

Ankur Midha (Poster Po-23 / Flash talk-2)

Freie Universität Berlin (FU), Berlin

The microbiome of the intestinal parasitic nematode *Ascaris suum* is derived but distinct from its host

Intestinal roundworms affect more than 1 billion people globally and are a major issue in animal husbandry. *Ascaris suum* is a zoonotic roundworm and a major problem in conventional pig farming. These pathogens live in intimate contact with the host gut microbiota and harbor bacteria within their own intestines. However, data on the parasite microbiome is effectively non-existent. In this study, we sought to characterize the microbiome of the parasite with respect to its environmental niche within the host. The intestinal microbiome of *Ascaris suum* was analyzed in contrast to the diversity and composition of the infected host gut. 16S sequencing of the parasite intestine and host intestinal compartments showed that the parasite gut has a significantly less diverse microbiome than its host and the host gut exhibits a reduced microbiome diversity at the site of parasite infection in the jejunum. While the host's microbiome composition at the site of infection significantly determines the microbiome composition of its parasite, microbial signatures differentiate the nematodes from their hosts as the *Ascaris* intestine supports the growth of microbes that are otherwise under-represented in the host gut. Our data clearly indicate that a nematode infection reduces the microbiome diversity of the host gut and that the nematode gut represents a selective bacterial niche harboring bacteria that are derived but distinct from the host gut.

Virginia Rossow (Poster Po-24)

Experimental and Clinical Research Center (ECRC-MDC/Charité), Berlin

OrkambiKIDS - Microbiome of CF-children

In the last decade a new class of medications became available for people with CF - CFTR-Modulators. They are able to directly influence the CFTR-Channel, which is impaired in function due to the underlying genetic mutation in the cystic fibrosis trans-membrane conductance regulator (CFTR) gene. Many studies have been conducted on this topic already, however most did not take the drugs' possible impact on the microbiome into account. Furthermore, when they were first released the modulators were only approved for people over the age of 12. Since 2018 children from 5 to 12 are allowed to be treated with Orkambi (Lumacavtor/Ivacaftor). OrkambiKIDS starts at exactly this point, as it follows eight children aged 5 to 12 for the first two years of treatment with Orkambi. On each of the nine visits throat, sputum and stool samples have been taken along with the corresponding clinical metadata like weight, height and lung function. Additionally, the children and their parents answered a questionnaire regarding the impact of the medication on their everyday lives. The samples were processed via 16S-rRNA sequencing and are currently being statistically analyzed. So far our interim results show that there is no uniform trend in how the microbiome evolves over the treatment. The results cluster by patient and not by time point. While measures like evenness and richness have no clear trend, for better or worse, at the end of the study period they generally do not fall under the baseline. Deeper insights will follow as the analysis progresses.

Hendrik Bartolomaeus (Poster Po-25 / Flash talk-4)

Experimental and Clinical Research Center (ECRC-MDC/Charité), Berlin

In-depth spatial analysis of microbiome-immune interaction in mice

The intestine harbors the largest immune cell reservoir. While many studies focus on specific immune cells, a comprehensive, segment-specific analysis of immune cells and microbes is lacking. We analyzed spatial microbiome-immune interaction using colonized (COL) and germ-free (GF) mice. Healthy COL and GF C57BL/6J mice (12 weeks) were sacrificed. Starting at the duodenum, intestines were divided into 5 segments and analyzed for local microbiome, intraepithelial (IEL) and lamina propria leukocyte (LPL) composition by shotgun sequencing, flow cytometry and immunofluorescence, respectively. Associated lymphoid organs (Peyer's patches, mesenteric lymph nodes), spleen and liver were included. In line with previous findings, a marked increase in absolute bacterial load from oral to aboral, with an accompanied increase in species diversity, was noted showing a spatial-dependence of features. Conversely, functional redundancy is seen across the different segments of the GI tract, as assessed by functional modules (KEGG/KOs), yet, this functional redundancy arises from unique genes across the segments (corroborated by the increasing species diversity). Immune cell composition varied across the intestine. Absolute numbers of LPL and IEL decreased from oral to aboral, with increasing relative proportions of adaptive immune cells. GF showed a reduced epithelial and LP area. Immune cell composition of GF or COL varied significantly, with T cell subsets being most affected. IL-17A producing cells (Th17 and ILC3) were almost depleted in GF mice. Absolute T cell counts were reduced in GF; aborally to a greater extent. Despite low bacterial loads in the small intestine, immune cell composition of COL was altered compared to GF. Systemic organs distant to the intestine were less affected by colonization status. In our study we could show that microbial functions are preserved across the GI tract, while diversity exists at the taxonomic and genomic level. Immune cell composition is segment-specific and dependent on the local bacterial colonization. Our study provides a tissue-specific insight and serves as a valuable resource for the scientific community.

Rima Hayani (Poster Po-26)

Freie Universität Berlin (FU), Berlin

Investigating the Microbiome of the Helminth Parasite Ascaris suum by Fluorescence in situ Hybridization

Parasitic nematodes infect over a billion people and are highly prevalent in wild, livestock, and companion animals. Ascariasis is the most prevalent helminth infection of humans and a major economic burden in conventional pig farming. Ascaris suum lives the majority of its life in the intestine of its host, surrounded by microbes. Furthermore, Ascaris itself has an intestine which is colonized by bacteria. However, interactions between Ascaris and the microbes in and around the worm are still poorly understood. The purpose of the current study was to study the microbiome of A. suum with a particular emphasis on the basic composition of the parasite microbiome and its location within the nematode intestine. We used Fluorescence in situ hybridization (FISH) to visualize bacteria along the intestines of adult A. suum worms obtained from a local slaughterhouse. The worms were cut in cross sections, fixed, embedded, and then a fluorescent probe for the 16S rRNA gene was applied. The sections were scanned using fluorescence microscopy and bacterial cells were counted. Preliminary findings indicate that live bacteria can indeed be visualized inside the intestines of adult Ascaris worms. We were only able to detect live bacteria within the intestine while all other tissues appeared to be free of bacteria. Furthermore, female worms appear to harbor more bacteria than male worms. Ongoing work is underway to identify the visualized bacteria by sequencing of the 16S rRNA gene.

Tobias Goris (Poster Po-27 / Flash talk-5)

Deutsches Institut für Ernährungsforschung (DIfE), Potsdam

Transformation of flavonoids by human gut bacteria

The metabolism of flavonoids, plant compounds which positively affect human health, in the human gut is still widely unknown. To shed more light on gut bacterial flavonoid conversion, we screened the Unified Human Gastrointestinal Genome collection (UHGG) including nearly 300,000 bacterial genomes for potential flavonoid-modifying enzymes by using sequences from known flavonoid-transforming enzymes as queries in a protein sequence similarity search. The potential flavonoid-modifying enzymes were quantified and observed to be highly abundant in bacteria not yet considered as flavonoid-converting species. For example, daidzein-to-equol converting enzymes, well studied in Slackia isoflavoniconvertens, were encoded only rarely by Slackia , but instead by uncharacterized Eggerthellaceae species with no isolates in strain collections. Of all potential flavonoid modifications, O-deglycosylation was by far the most abundant. Putative C-deglycosylating enzymes were encoded less often; mainly in Agathobacter (formerly Roseburia) faecis. Enzymes involved in flavonoid degradation, such as ring-cleaving reductases or phloretin hydrolases were mainly detected in Flavonifractor plautii, a bacterium recently suggested to be correlated in colorectal cancer development in India. We tested three bacteria for their ability to convert flavonoids, including a novel Catenibacillus strain that encodes the highest number of putative flavonoid-modifying enzymes observed until now. All three species exhibited flavonoid-modifying activities, such as derhamnosylation, C-deglycosylation or degradation. This combined in silico/physiological insight into the flavonoid metabolism of the human gut highlights overlooked bacterial species as potential key organisms in flavonoid conversion in the human gut.

Aline Rosin (Poster Po-28)

Bundesinstitut für Risikobewertung (BfR), Berlin

Establishment of a commensal 3D-skin model for melanoma progression studies

The junior research group for microbiome research focuses on the interaction of commensals with their host. Previously, a co-culture system was developed comprising a microbially competent three dimensional (3D) skin model for modulation of the toxicity of chemicals. This approach is now to be extended to a melanoma model. The human skin is one of the largest and most versatile organs of the human body. It harbours millions of microorganisms, namely bacteria, fungi and viruses. Together these are referred to as the skin microbiome. Particularly the bacteria are involved in many cellular processes like pathogen protection, wound healing and immune modulation. Dysbiosis refers to a lack of balance among bacterial communities and the host that may lead to skin diseases. Recent studies showed that dysbiosis also occurs in melanoma, the black skin cancer. However, the underlying mechanism of melanoma progression and skin microbiome is not yet sufficiently understood.

The participant could not attend but agreed to include the work in this booklet

Annika Oßwald (Poster Po-29)

Deutsches Institut für Ernährungsforschung (DIfe), Postdam

Deoxycholic acid promotes colonic tumorigenesis in gnotobiotic mice colonized with a bile acid-converting simplified microbiota

The secondary bile acid deoxycholic acid (DCA) is produced by human gut bacteria and was shown to be positively correlated with colorectal cancer (CRC). Mechanisms underlying the tumor-promoting function of DCA are still not clear and the effect of in vivo produced DCA on CRC development was not demonstrated to date. Here we investigate the tumorigenic effects of DCA in the colon using gnotobiotic mice colonized with a simplified microbial consortium. We further analyze the effects of the presence/absence of DCA on markers associated with CRC, bile acid metabolism and intestinal regulatory T-cells (Treg). Germ-free wildtype mice were colonized with a simplified bile acid-converting microbiota with (BACOMI) or without (BACOMI-S) Clostridium scindens, which has a 7-adehydroxylating activity, resulting in DCA production. A subgroup of BACOMI colonized mice was treated with AOM/DSS to induce colonic tumorigenesis. Bacterial abundance in colon content was measured using qPCR and bile acids were quantified by LC-MS/MS. In the colon, expression of genes related to tumorigenesis and bile acid metabolism were analyzed using qPCR and proliferation of epithelial cells assessed by Ki67 staining. Treg were isolated from colon and iliac lymph nodes and analyzed using flow cytometry. Germ-free wildtype mice were successfully colonized with the microbial consortium and DCA was only present in mice colonized with BACOMI but not in BACOMI-S mice lacking C. scindens. In mice treated with AOM/DSS, colonization with BACOMI led to higher numbers of colonic tumors compared to mice colonized with BACOMI-S. Even without chemically induced tumorigenesis, CRC-associated genes (e.g., Ccnd1, Ptgs2 and Myc), as well as genes involved in bile acid metabolism (e.g., Slc10a2, Nr1h4 and Slc51b) were upregulated in the colonic mucosa of BACOMI compared to BACOMI-S colonized mice. BACOMI mice had higher numbers of Ki67 positive cells in the colon and showed reduced colon lengths compared to BACOMI-S mice. Foxp3+, Foxp3+ CD103+ and Foxp3+ CD304+Treg were more abundant in the iliac lymph nodes of BACOMI compared to BACOMI-S mice, but no differences in these T-cell populations were detected in the colon. Colonization of germ-free mice with different BACOMI consortia is a suitable model to investigate the effects of DCA on colonic tumorigenesis and intestinal immune cells in vivo. The presence of DCA in this gnotobiotic model system leads to increased levels of different mucosal markers associated with CRC and higher numbers of colonic tumors, demonstrating the tumor-promoting function of DCA.

Rebecca Luise Knoll (Poster Po-30 / Flash talk-6)

Experimental and Clinical Research Center (ECRC-MDC/Charité), Berlin

Patterns of gut microbial dysbiosis despite probiotic supplementation at day 28 of preterm life

Background: Preterm birth is associated with particular challenges for gut microbiome maturation. Preterm newborns are delivered in 90% of cases via C-section and they receive in 75% of cases antibiotics during their first days of life. They are exposed to the hospital environment for a much longer period of time, then fullterm newborns. Together these factors facilitate development of gut microbiome dysbiosis. This gut dysbiosis might increase the risk of infections as sepsis and necrotizing enterocolitis and/or colonization with multi-drug-resistant bacteria. Methods: The PRIMAL randomized-controlled clinical trial investigated whether the probiotic supplementation with Bifidobacterium infantis+longum and Lactobacillus acidophilus (Probactiol infantis®; Metagenics, Netherlands) daily for the first 28 days of life can prevent detection of gut dysbiosis at day 28 and leads to decrease of infection incidence in those supplemented preterm newborns. A total of 646 preterm infants were enrolled and randomized in the clinical trial (323 each arm). Stool sampling was performed at day 1, day 28 and day 365 of life. In addition, 300 mothers provided a stool sample at day of giving birth. As a control group, 100 fullterm healthy breastfed newborns, provided stool samples at day 1, day 28 and day 90 of life. DNA was extracted (Qiagen PowerSoil Kit) and 16S rRNA gene sequencing performed on an Illumina Miseq targeting the V4 region (249 bp, forward and reverse). Samples were discarded from further analyses when they had < 500 total reads, for subsequent analyses relative abundances were used. Alpha-diversity of microbiomes and relative abundances of KRINKO-reported pathogens and key gut microbiome genera were compared between sample groups. Results: 2283 samples were analyzed. In comparison to the fullterm healthy microbiome, the preterm microbiome displayed lower alpha-diversity measures at day 28 of life (Kruskal-Wallis, fdr <0.000), but not at day 1. At day 28 preterm newborns displayed higher abundances of the genera: Clostridium sensu stricto 1, Enterococcus, Klebsiella, and Staphylococcus. In contrast Bacteroides, Faecalibacterium and Streptococcus were decreased in abundance. (MWU, fdr <0.000). Analyzing the abundance of the probiotic supplemented genera, Bifidobacterium was significantly enriched in the preterms, but Lactobacillus displayed lower abundances (MWU, fdr <0.05). Subsequent analyses between probiotic and placebo treatment are still pending. Conclusion: Despite the fact that treatment allocation is still blinded and differences in probiotic supplemented and non-supplemented preterm microbiomes were not investigated, significant differences between the preterm and the fullterm microbiome composition were observed. This proves altered gut microbiome composition in preterm newborns at day 28 of life. Future analyses will disentangle whether probiotic supplementation is protecting the supplemented preterm newborns at least partially from developing dysbiosis and if dysbiotic gut microbiome patterns can still be observed at day 365 of life.

Jonathan Riedmüller (Poster Po-31)

Institut für Tierernährung, Freie Universität Berlin (FU), Berlin

Zinc supplementation in weaning piglets: A comparison of source and concentration

The supplementation of high levels of zinc oxide in the diet of weaning pigs has been a common practice in the swine industry for many years. However, with the upcoming regulations regarding zinc oxide use, scientists, farmers, and companies are bound to investigate new solutions for maintaining piglets gut health and performance. Approaches to solve this issue often include the use of more efficient sources of zinc, which could be included in lower levels while keeping similar growth performance and health of piglets. The objective of this study was to investigate the effects of graded zinc concentrations from a potentiated zinc source (HiZox) compared to a standard zinc oxide on growth performance, fecal score, and enterobacteria of weaned pigs. A total of 1440 weaned pigs were randomly allotted to 12 treatment groups (144 pens, 12 pens per treatment, and 10 piglets per pen). Piglets were fed either the potentiated zinc source (HiZox) or the standard ZnO in the follow levels per kg of feed: 150, 300, 600, 900, 1500, or 3000 mg of zinc. The piglets were fed treatment diets for two weeks, after that all groups from both zinc sources were fed the same diet containing 150 mg/kg of Zn from HiZox. Body weight (BW) and feed intake per pen were obtained weekly. From these data, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated. On day 14, pools of feces were collected from pens where pigs were fed 150, 600, 1500, or 3000 mg/kg of Zn from both sources, to investigate the gene frequency of pathogenic E.coli isolates. To extract the DNA from the feces the QUIAGEN "QIAamp® PowerFecal® Pro DNA Kit" was used. Extracted DNA was quantified using the Quantus fluorometer (Promega) with QuantiFluor®. Then, faecal *E.coli* isolates were identified, as well as the 2 adhesin and 2 toxin gene sequences: fae, fedA, est-II, and est-IB, which encode the K88 adhesin (F4), the F18 fimbrium, stable enterotoxin II, and the heat stable enterotoxin I, respectively. SPSS (Version 26, IBM, USA) was used to perform an analysis of variance (ANOVA) and a post-hoc test (Tukey HSD). Differences were considered statistically significant when $p < 0.05$. A 16S-Gene-Sequencing has also been performed and the analysis of these results is still on going. Piglets were healthy and had no diarrhea issues during the period of the trial. The ADG was higher ($P < 0.01$) on pigs fed potentiated Zn at 300, 600, and 900 mg/kg of Zn compared to pigs fed ZnO at the same dose. The ADFI was higher ($P<0.05$) in pigs fed potentiated Zn at 300 and 600 mg/kg of Zn compared to pigs fed same levels of Zn from ZnO. Finally, FCR was higher ($P<0.05$) for pigs fed 300 mg/kg of Zn from potentiated Zn than from ZnO. Other doses comparisons were not different between Zn sources ($P>0.05$). At day 14 after weaning, when comparing 3000 mg/kg of Zn from ZnO with 150 or 300 mg/kg of Zn from potentiated Zn, piglets' performance was not different ($P>0.05$). The *E. coli* isolates derived from weekly fecal samples of nursed piglets were characterized regarding the occurrence of 4 pathogenicity genes (i.e., 2 toxin and 2 fimbriae genes). Colony-forming units of total fecal *E. coli* were comparable between treatment groups. Pigs fed 600 mg/kg of Zn from HiZox presented the higher level of isolate carrying pathogenicity genes estII compared to other groups, except for pigs fed 1500 mg/kg of Zn from ZnO. The occurrence of the two fimbriae genes (fae and FedA) did not differe between groups. At day 14 after weaning, when comparing 3000 mg/kg of Zn from ZnO with 150 or 300 mg/kg of Zn from potentiated Zn, piglets' performance was not different ($P>0.05$). Analyses related to gut microbiota from this study are still in progress. In conclusion, in a healthy environment, potentiated Zn may be an alternative to the pharmacological level of regular ZnO.

Carolina Schwedhelm (Poster Po-32)

Max-Delbrück Center for Molecular Medicine (MDC), Berlin

Differences in the gut microbiome composition between individuals with or without obesity – Preliminary results from federated analyses in the context of the Knowledge Platform Intestinal Microbiomics (INTIMIC-KP)

Background: Differences in the gut microbiome composition in obesity versus non-obesity have been observed in human studies, although systematic literature reviews and meta-analyses observed substantial differences in findings across existing studies, and no consistent taxonomic signature of obesity has been identified. This may partly be related to heterogeneity in methodology across studies as well as diverse body mass index (BMI) comparison groups and the lack of adjustment for potential confounding factors (many studies did not even adjust for age and sex). The joint analysis of studies based on harmonized individual-level data may overcome some of these issues. Objectives: The aim of this study is to examine differences in alpha diversity as well as gut microbiome composition at the phylum and genus level in adults with or without obesity in a joint federated analysis of harmonized data from multiple European observational studies participating in the Knowledge Platform Intestinal Microbiomics (INTIMIC-KP). Methods: Cross-sectional data from seven European observational studies and from the control arm of one intervention study were harmonized according to a joint study protocol. Obesity was defined as measured BMI >30. Using DataSHIELD in R, joint federated virtual individual personal data (IPD) analysis and study-level meta-analysis (SLMA) using generalized linear models were conducted without the need to physically pool or share data. Results: Data on 7,577 participants (56% female, 18-79 y) from six studies were available for preliminary analyses. One study used shotgun metagenomic sequencing for microbiome characterization, while the other five studies used 16S rRNA gene sequencing with varying amplified regions. Age, sex, and study source-adjusted IPD models showed lower alpha diversity (Shannon index) in adults with versus without obesity (β= -0.15; 95%CI= -0.18, -0.13). While SLMA results were consistent, it revealed substantial heterogeneity across studies (I2=70%). Conclusions: Preliminary results from this joint federated analysis support the hypothesis of decreased alpha diversity in adults with obesity. While data harmonization reduced variable-related heterogeneity, substantial heterogeneity remained. A higher degree of standardization and partial removal of technical biases may be reached by running a common bioinformatics pipeline.

Birgit Walther (Poster Po-33)

Robert Koch Institut (RKI), Berlin

Perioperative antibiotic prophylaxis (PAP)-induced changes of the gut microbiota in horses elicit a common trajectory

Johannes Schulze Holthausen (Poster Po-34)

Freie Universität Berlin (FU), Berlin

The effect of glutamine supplementation, birth weight and age on the dominant microbial composition in the stomach of suckling piglets

Introduction. Knowledge on the effect of glutamine (Gln), birthweight and age on microbial composition in the stomach of suckling piglets is scarce. Therefore it was the aim of this study to examine their influence on the stomach microbiome of suckling piglets. Methods. At birth (d (day) 0), male low (LBW) and normal (NBW) birthweight piglets were selected, and orally supplemented with Gln or an isonitrogenous amount of alanine (Ala) from d 1 until 12. Four different groups were studied: LBW+Gln; NBW+Gln; LBW+Ala; NBW+Ala (n = 5/group/age-group). Subgroups were euthanized at d 5 and 12 and stomach digesta snap frozen and stored at -80 °C. DNA was extracted from the digesta samples and metagenomic sequences were determined. Kruskal-Wallis-Test was performed to detect dominant bacteria influenced by supplementation, birthweight and age ($p < 0.1$). Results. The main abundant bacteria in stomach (> 2 %) at d 5 and 12 belonged to the genus Lactobacillus. Glutamine supplementation increased the abundance of *Lactobacillus mucosae* ($p < 0.05$) and the abundance of *Lactobacillus amylovorus* whereas Gln decreased the abundance of *Lactobacillus crispatus* ($p < 0.1$) compared to Ala supplemented piglets. Birthweight had no effect. With increasing age the abundance of Lactobacillus amylovorus and mucosae decreased ($p < 0.05$) whereas the abundance of *Lactobacillus crispatus* and *reuteri* ($p < 0.001$) and the abundance of *Lactobacillus vaginalis* increased ($p < 0.1$). Conclusion: Glutamine supplementation had minor effects and age had the most effects, on the main stomach microbial composition in suckling piglets.

Jannike Krause (Poster Po-35 / Flash talk-7)

German Rheumatism Research Centre Berlin (DRFZ), Berlin

Sample logistics affect structural and functional profiles of faecal microbiota

Many aspects in the crosstalk between the intestinal microbiota and its host is mediated by soluble metabolites, which can modulate cellular differentiation and function. However, the metabolome, comprising all low molecular weight compounds, is prone to quickly alter upon environmental changes. This poses a challenge to sample logistics and subsequent ex vivo analysis. Here, we investigated the impact of time between defecation and analysis, oxygen exposure and storage condition on the composition and metabolome of faecal microbiota to determine most optimal conditions of sample logistics for analysis of microbial functionality. Faecal samples of six healthy individuals were put under anaerobic conditions directly after defecation and processed within 30 min for storage under different conditions: native at room temperature, 4 °C or -20 °C and -20 °C 1:10 diluted in 12.5% glycerol, both in the presence and absence of oxygen. Samples were analysed after 4 h, 24 h, 48 h and 168 h of storage. The microbiota were analysed (i) by microbiota profiling using microbiota flow cytometry (MFC) to assess changes in community structure and (ii) by short-chain fatty acid (SCFA) profiling using LC-MS/MS to determine changes in community functionality. Hierarchical clustering of all samples revealed a donor-dependent clustering of MFC- and to a lower extent SCFA-profiles. To determine the effect of the different storage conditions, we calculated the Bray-Curtis (BC) similarities comparing the profiles of stored samples to the “fresh” sample for each donor. With increasing storage time, the BC similarity decreased, showing a significant negative correlation of BC similarity and time. Interestingly, our data reveal that the degree of storage-dependent change is donor-dependent, an important aspect that needs to be considered when comparing many individuals to each other. The different storage conditions as well as oxygen exposure affected MFC and SCFA profiles in different manner. Storage as native faecal sample at 4 °C or in 12.5% glycerol at -20 °C for up to 24 h best conserved MFC- and SCFA-profiles compared to the fresh faecal sample. Global metabolomics using LC-MS/MS and functional bioassays will be performed in the near future to complement our understanding on how logistics of faecal samples affects functionality.

Amelie Weber (Poster Po-36)

Charité Universitätsmedizin, Berlin

Microbial energy in immune homeostasis and function

Energy provided by microbially fermented dietary fiber can account for up to 10% of a person's total energy. However, the effects of this energy contribution to the host's physiology are poorly understood.
We hypothesize that energy provided by the microbiota is crucial in the regulation of the immune cell homeostasis and function. To test this hypothesis, we are currently performing an in-depth metabolic analysis of germfree mice.
Furthermore, using flow cytometry we have found that in germ-free mice the egress of monocytes from the bone marrow is strongly limited resembling what is observed in fasting mice. The decrease of monocyte numbers was not due to decreased differentiation of monocyte progenitors in the bone marrow. Of note, we have made similar observations in mice fed with a diet composed of non-fermentable fiber,
Our findings indicate that not only direct immune signaling, or microbial metabolites affect immune homeostasis but also the energy that is provided to the host through microbial fermentation.

Lisa Budzinski (Poster Po-37)

German Rheumatism Research Centre (DRFZ), Berlin

Multi-parameter flow cytometry identities distinct microbiota phenotypes in chronic inflammatory diseases

A hallmark of chronic inflammatory diseases (CID) is an alteration of the intestinal microbiota, also called dysbiosis. Experimental animal models strongly suggest that dysbiosis contributes to the disease. In clinical studies microbial 16S rRNA gene profiling by next generation sequencing has greatly contributed to our understanding of taxonomic alterations of the microbiome in disease, but has failed so far to conclusively identify bacteria or bacterial communities contributing to disease pathogenesis. We are using multi-parametric flow cytometry to analyze the human intestinal microbiota from stool samples on the single cell level and assess phenotypic properties of the bacteria, which may be important for the microbe-host interaction. We analyze the coating of patient´s intestinal microbiota by isotype-specific staining of host immunoglobulins to capture the immunological context of their recognition by the host. In addition, we characterize microbial surface sugars with specific lectins, which may indicate metabolic conditions, adhesive ability and bacteria-host-crosstalk. Using our method, we can discriminate distinct microbial community phenotypes in patients with different chronic inflammatory diseases (Crohn´s disease, ulcerative colitis, IgG4-related disease, juvenile idiopathic arthritis, rheumatoid arthritis). Applying machine-learning approaches, we can delineate phenotypic clusters that allow robust classification of disease entities. This approach suggests that we can use multi-parametric microbiota flow cytometry of stool samples for diagnosis and disease-monitoring but also to identify intestinal microbial communities specific for certain diseases and potentially playing a role in disease pathogenesis.

Aayushi Shah (Poster Po-38)

German Rheumatism Research Centre (DRFZ), Berlin

Understanding the role of microbiota populations in licensing of pathogenic T cells in intestinal bowel diseases

The intestinal health is reflected by the composition and the diversity of the microbes that reside in it. In state of intestinal bowel diseases (IBD), there is most often a dysbiosis that occurs, causing either an enrichment or decline of certain bacterial populations, thus there is a need to define and understand these changes in the microbiota and how it translates to the changes in immune compartment ultimately leading to chronic inflammation as in the case of IBD.
To understand this crosstalk between the bacterial populations and immune system, we performed single cell and TCR sequencing of the intestinal lamina propria CD4 T cells and intestinal epithelial cells post T cell transfer colitis in Rag1-/- deficient mice with naive T cells from different genotypes (WT, Tbx21-/-, RORc-/-, Tbx21-/- RORc-/-). The result shows certain effector T cells cluster shared across all genotypes and certain clusters unique to each genotype. Additionally, the TCR sequencing shows oligoclonal expansion of TCRs indicating the T cell response to specific bacteria. We aim to associate these expanded TCRs to the bacteria by cloning the expanded TCRs into a T cell line and subsequently screen the compartments of microbiota obtained by sorting the bacteria through flow cytometry. With this we want to dwell deeper and identify which populations in the microbiota contribute to a certain T cell phenotype. Furthermore, we also aim to investigate effect of intestinal epithelial cells in role in licensing of T cells by looking into adaptation markers, change in cytokine and chemokine expression.
Understanding this crosstalk between the pro- and anti-inflammatory bacteria and the Th cells could contribute to a better understanding of the development of chronic inflammatory bowel diseases.

Anika Hartmann (Poster Po-39)

Charité Universitätsmedizin, Berlin

Rheumatoid arthritis benefits from fasting and plant-based diet: an exploratory randomized controlled trial (NutriFast)

Fasting has been shown to be beneficial in many diseases, including rheumatoid arthritis (RA). Among other effects, fasting stimulates ketogenic metabolism, induces autophagy, and harbors immunomodulatory functions. Recent studies have highlighted the role of the intestinal microbiota in the still unclear etiology of RA[1]. This could be a potential target for additional dietary therapy in RA. To investigate the effect of therapeutic fasting followed by a plant-based diet compared to standard dietary recommendations in patients with RA. In this pilot study patients with RA were randomized to either a 7-day fast (≤250kcal/d) followed by 11 weeks of plant-based diet or to conventional nutritional counselling according to the recommendations of the German Society for Nutrition (Deutsche Gesellschaft für Ernährung, DGE) for 12 weeks. Disease activity and treatment response in RA (including Health Assessment Questionnaire, HAQ; EULAR Response Criteria, ACR Response Criteria) were measured at baseline (T0), day 7 (T1), 6 weeks (T2) and 12 weeks (T3). A total of 50 from 53 enrolled participants were included into the per-protocol analysis. The mean age was 51.98 ± 9.4 years with symptoms duration of 6.8 ± 8.1 years; 92% were females and 78% were ACPA and/or RF IgM positive. At baseline, participants presented HAQ 0.8 ± 0.5, DAS28CRP 4.0 ± 1.3, CRP 3.1 ± 3.8 mg/L, and a BMI of 25.0 ± 3.7 kg/m^2. The primary endpoint did not become significant. However, post-hoc analyses revealed clinically relevant improvements in the HAQ after 12 weeks in both the fasting and the DGE group (Δ-0.29; 95% CI, -0.45 to -0.13; p = 0.001; and Δ-0.23; 95% CI, -0.45 to -0.22; p = 0.032; respectively). Furthermore, the effect already set on by day 7 in the fasting group compared to week 6 in the DGE group. This effect was independent of antibody status, delivery mode of the intervention or previous dietary forms. CV risk factors including weight and total cholesterol levels improved stronger in the fasting group compared to the DGE group (Δ-3.9 kg vs. -0.7kg; 95% CI, 1.4 to 5.0; p = 0.001 and Δ-18.60 mg/dl vs Δ6.44 mg/dl; 95% CI, 7.3 to 42.8, p=0.007).

Carola Ellner (Poster Po-40)

Freie Universität Berlin (FU), Berlin

Effects of dietary rye and rapeseed on microbiota and electrophysiological parameters of the jejunum in weaner pigs

Rye and rapeseed meal (RSM) are alternatives to wheat and soybean meal (SBM) in pig nutrition. The fibre composition of feed can affect intestinal microbiota and nutrient absorption (Agyekum 2017). Rye contains more soluble fibre than wheat, RSM more insoluble fibre than SBM (Bach Knudsen 2014). This study aimed to investigate the effect of rye and RSM on microbiota and electrophysiological parameters in the jejunum of piglets. 88 weaner piglets were allocated to 44 pens receiving four diets over five weeks: wheat/SBM, wheat/RSM, rye/SBM or rye/RSM. Diets contained cereals at 48%, RSM at 30%, SBM at 25% and were analysed for fibre composition. A dissection served for sampling of jejunal digesta and tissue. Microbiome was analysed via 16S rRNA sequencing. Tissue was assessed for basal tissue conductance and the change of basal short-circuit current. Statistics were carried out using a 2-factorial ANOVA with cereal (CER) and protein meal (PM) as fixed factors ($p<0.05$). Rye-based diets contained more SDF than wheat, RSM-based diets more IDF than SBM. Rye increased relative abundance of Firmicutes ($p = 0.039$), decreased Proteobacteria ($p = 0.002$). RSM increased Proteobacteria ($p = 0.019$) and Actinobacteria ($p = 0.019$), decreased Firmicutes ($p = 0.004$). Electrophysiological parameters of tissue were not affected. This study shows that rye shifts jejunal microbiota towards carbohydrate degrading Firmicutes and reduces Proteobacteria containing putative pathogens. RSM reduced Firmicutes which might be due to higher IDF content. However, alterations of resident microbiota seemed not related to transport physiology of the gut wall.Abstract missing.

Isabel Dorst (Poster Po-41)

BIOMES NGS GmbH, Berlin

Beyond association and correlation: Data mining based on real world microbiome profiles

Microbiome studies have identified a variety of intrinsic and extrinsic factors associated with specific patterns of the gut microbiome. However, most large microbiome datasets display a lack of methodological consistency and usually examine only one microbial snapshot, thereby neither representing intraindividual dynamics over time nor allowing causal inferences. Moreover, there is no real-world evidence that accurately reflects circumstances of daily life and natural consumer behavior. This study provides insights into typical application of BIOMES' complex, heterogeneous and population-based microbiome data, based on a simulated dataset, leveraging 16S rDNA amplicon sequenced DNA and metabolic pathway prediction. By using up to 80 variables of BIOMES metadata on environmental, nutritional, lifestyle, and anthropometric factors and personal medical history, a multivariate and compositional analyses is conducted. The impact of grouped lifestyle factors on the gut microbiota is quantified, identifying general nutrition as the most influential factor. Significant links are identified by correlating dietary indices with genus abundances. Stratified longitudinal analysis is pursued for one highly correlated taxon, suggesting a putative causal relationship between dietary fiber consumption and the investigated genus. This illustrates how BIOMES dataset of over 30,000 microbiome profiles are used for exploratory purposes, hypothesis generation, and hypothesis validation. Cross-sectional and longitudinal real-world data can be used to establish correlations and possible causality to address the current gap in microbiome research.Abstract missing.

Noushin Arfatahery (Poster Po-42)

Freie Universität Berlin (FU), Berlin

Identification of *Vibrio kanaloae* in the oyster *Crassostrea gigas* by fluorescent in situ hybridization

An ideal model for studying *Vibrio* and host and their interaction in disease dynamics is *Crassostrea gigas* in the North Sea, exposing invasive sources to pathogens, especially (*Vibrio kanaloae*). This study used molecular fluorescent in situ hybridization (FISH) techniques to rapidly identify the diversity of bacteria in the oyster Crassostrea Gigas from Sylt Island and 16SrRNA gene sequence validated all strains' identification. Oysters were collected and exposed with *Vibrio kanaloae* strains on different tissues were examined utilizing culture-independent methodologies. The digestive glands, gill and muscle in *Vibrio kanaloae* were identified as metabolically active by the FISH technique, one of the advantages of the FISH technique is that it is accurate and very easy to use. This technique allows rapid assessment of *Vibrio* in oysters and seafood.

Sonja Wende (Poster Po-43/Flash talk-3)

Leibniz Zentrum f. Agrarlandschaftsforschung (ZALF), Berlin

Functional Metagenomics as a tool to decipher crop microbiomes

Crop plants and their microbiota are considered as an ecological functional entity that jointly adapts to environmental stressors. Wheat (*Triticum aestivum* L) is crucial for humanity's nutrition as it has a global annual yield of more than 770 Mio tons. Climate change-associated extreme weather events such as heavy rain events and drought periods have increased in frequency over the past decades and are predicted to increase further. Flooding and drought lead to deleterious changes in the water availability and flooding causes harmful oxygen limitation. In previous studies, often the focus has been laid on the structurally taxonomic changes of the microbiota. Here, we chose a metagenome approach to assess functional adaptations of the bacterial microbiota of wheat to flooding and drought in a glass house experiment with the variety Chinese Spring. This work is corroborated by an accompanying published study in which we resolved structural responses by metabarcoding (Francioli et al. 2021). At three growth stages (tillering, flowering, ripening) we conducted three treatments, i.e flooding, drought and a well-watered control. We sampled and analysed per growth stage the rhizosphere microbiota by high through-put sequencing of DNA. We employed diamond and MEGAN6 for annotation and subsequent analysis of the read datasets.Further, we annotated both, MAGs and metagenomic reads, with PGPT-Pred (in revision Patz et al. 2021) that uses an ontology built on about 6,900 hierarchically organized plant growth promoting traits (PGPT), and a recently established mgPGPT-db for MEGAN6 (preprint Patz et al. 2022). The analysis of PGPTs with MEGAN6 revealed shifts of metabolic functions of the bacterial rhizosphere microbiota regarding oxidative stress regulation, anaerobic respiration, temperature resistance, and the occurrence of mobile elements. MAGs analysis further provided genomic structural regions associated to PGP functions apparent in response to abiotic stressors. Hence, a concerted enrichment of PGP-traits in the bacterial community was observed when wheat was grown under water stress.

Víctor Hugo Jarquín Díaz (Poster Po-44)

Experimental and Clinical Research Center (ECRC-MDC/Charité), Berlin

Revealing the patterns of bacteria and antimicrobial resistance from the gastrointestinal ecosystem in wild populations of house mice

Antibiotic resistance (AR) is a biological phenomenon emergent at multiple levels, and also a priority public health problem. Selection mechanisms for AR are well understood, while the transmission of antibiotic resistance genes (ARGs) is a comparatively under-researched topic. Particularly, the links between molecular, genomic, bacterial community, and host community level are rarely analyzed in an overarching manner.Given the importance of understanding how bacteria carrying ARGs interact in the microbiome and with the environment of their hosts, here we used an amplicon sequencing approach, to simultaneously study bacteria and predict ARGs composition in natural populations from house mice (*Mus musculus*). We compared gastrointestinal bacterial diversity, composition and abundance across a gradient of pure and hybrid genotypes in the European house mouse hybrid zone between the subspecies *M. m. musculus* and *M. m. domesticus* at different geographical and temporal scales. We detected transgressive phenotypes of abundance linked to the genotypes. For some bacterial and ARGs, they tend to exceed abundance in hybrid genotypes. In contrast, the abundance of other bacteria are reduced in hybrids compared to parental mice. Our results confirm that host genotype drives the abundance of gastrointestinal bacteria and potentially also ARGs in natural populations of house mice.

Łukasz Grześkowiak (Poster Po-45)

Freie Universität Berlin (FU), Berlin

Influence of fiber composition in sows' diet on the colonization of gut pathogens in the offspring

Dietary fiber has a potential to modulate gut microbiota in sows. It was hypothesized that a maternal diet rich in either high- or low-fermentable fiber during gestation and lactation influences the colonization of gut pathogens in their offspring. Twenty sows were fed gestation and lactation diets enriched with either high-fermentable (SBP, 15% sugar beet pulp) or low-fermentable (LNC, 15% lignocellulose) fibers. Pathogenic bacteria (*Escherichia coli-Hafnia-Shigella, Streptococcus suis, Clostridium perfringens, Clostridioides difficile*), bacterial virulence factors (*C. difficile* toxins TcdA and TcdB) and segmented filamentous bacteria (SFB) were determined weekly in DNA from feces of piglets during the suckling period until two weeks post weaning using qPCR method. Significance was considered at $p \leq 0.05$. In 6-day-old piglets concentrations of C. difficile and TcdA were higher in animals from sows fed LNC vs. SBP. At 21 days of age, *C. perfringens* was higher in piglets form sows fed LNC vs. SBP. At weaning, DNA copy numbers belonging to *E. coli-Hafnia-Shigella* and *C. perfringens* were higher in piglets from LNC vs. SBP. Two days post weaning, higher counts of *C. perfringens* were detected in the feces of piglets from LNC vs. SBP. One week post weaning, S. suis was higher in piglets from SBP vs. LNC group. Two weeks after weaning, *E. coli-Hafnia-Shigella* was higher in piglets from LNC vs. SBP. Susceptibility to colonization by certain gut pathogens in piglets can be influenced by the sows' nutritional factors supporting the phenomenon of the mother-offspring early microbial programming. The significance of these findings requires further assessment.

Science slam

Lisa Budzinski (Science slam)

German Rheumatism Research Centre (DRFZ), Berlin

Horospoop

Bruno Rocha Cordeiro Costa (Science slam)

Charité Universitätsmedizin, Berlin

Wildling microbiota induces changes in the immune and structural cell compartments of the skin

The epithelial barrier of the skin is one of the largest border surfaces with the environment and it is exposed to microbes, light, irradiation and physical or mechanical stress. Innate lymphoid cells (ILC) are a recently identified group of innate lymphocytes with preferential representation at border surfaces, where they can closely interact with the microbiota. Two principal ILC lineages exist, killer or cytotoxic ILC (conventional NK cells) and helper- like ILC. Among helper-like ILC, three groups are discriminated (ILC1-3), transcriptional and effector programs of which share striking similarities with the various T helper cell subsets. Previous studies show that the microbiota is a potent inducer of ILC function in the gut and to investigate if they also effect dermal ILCs, we used the wildling mice mode, created to address shortcomings of current mouse models. Wildlings resemble wild mice and differ significantly from conventional laboratory mice in their bacterial microbiome at major microbial niches and immunological barrier sites. Our results suggest that wildling microbiota induces accumulation of leukocytes in the skin, with a specific increase in αβ T Cells, γδ T Cells and ILCs. All the mentioned lymphoid cell types have an increased cytokine production when compared to standard laboratory mice. The microbiota also affects the epithelium, leading to a higher expression of keratinocyte related genes and increased barrier thickness. Understanding how commensal microbiota dictates immune cell function and structural cells organization in a healthy state can potentially contribute to better therapies and outcomes for skin pathologies, such as atopic dermatitis, psoriasis or contact allergy.

Nizar Shayya (Science slam)

Charité Universitätsmedizin, Berlin

Colonization Resistance: a first line of defense

The author prefers that the abstract is not included in this book

Bhakti Irene Seth

Max-Delbrück Center for Molecular Medicine (MDC), Berlin

Gut-Brain modulation of hypertension

An association between gut dysbiosis and cardiovascular diseases like hypertension (HT) is already well established but the directionality of this axis is still under debate. Furthermore, the bidirectional gut-brain axis has several arms that branch out to impact the heart. The overarching objective of this project is to investigate a neural circuit between the gut and the heart, which is mediated by a microbiota-brain interaction that affects HT and its associated cardiac remodeling. To better understand this gut-brain-heart network, we employed a hypertensive model of zebrafish developed by our lab.

I first aimed to understand the gut-heart connection in our hypertensive model using germ free (GF) fish. We found that with the absence of conventional microbiota, the disease was exacerbated. We therefore now want to identify these “protective” microbiota that are absent in our GF fish, and ways to mirror their effect through the metabolites they produce. We also identified 2 hypothalamic populations that are upregulated in the disease. Upon functionally verifying these findings using immunohistochemistry, we aim to assess whether these neural clusters are also influenced by the gut upon hypertensive fecal microbial transplantation. This will allow us to establish a modulating neuronal circuit which mediate microbiome-driven regulation of HT and its associated cardiac phenotypes.

The participant could not attend but agreed to include the work in this booklet

Jenny Jaffe

Robert Koch Institut (RKI), Berlin

Challenges interpreting necrobiome and urinary microbiome in wild chimpanzees

Several microbiota were analyzed as part of research investigating wound healing and kidney disease in wild habituated chimpanzees (*Pan troglodytes verus*) in Taï National Park, Côte D'Ivoire. Samples were screened on the variable regions V3 and V4 of the bacterial 16S rRNA gene by PCR. For the study of wound healing, 16S PCR and sequencing was performed on internal organs and purulent wound tissue of two chimpanzees that died several weeks after severe trauma, likely caused by leopard attacks. Though histopathology of the wounds of the second chimpanzee showed predominance of Gram-positive cocci, the 16S results did not show predominance of a single family or genus, rather a mixed picture. The wounds and the spleen of this chimp showed presence of bacteria known to occur in the oral microbiome. For the so-called 'necrobiome' we can compare with earlier work on primates found dead in Taï National Park. Those findings show that in some cases, a predominant genus and likely cause of death can be pinpointed, whereas in other cases, 'classic necrobiota' can be seen, which are more likely overgrowth of non-pathogenic bacteria present in the carcass. Still, with limited comparative data, tropical climate and time between death and necropsy not exactly known in one the two cases, challenges interpreting the findings of these two cases remain. The second topic of study, kidney disease, was focused on an older female chimpanzee who was cachectic at time of death, and was found to have a large kidney stone on post-mortem examination. Histopathology and albumin/creatinine ratios in urine samples showed a history of chronic kidney disease. The kidney tissue and kidney stone extractions did not show clear bands on 16S PCR, indicating minimal presence of bacteria in these tissues. Spleen and liver tissue did show clear bacterial bands, and though initial analysis pointed towards *Clostridium* spp. as predominant bacteria, further analysis showed that these results were false positives. As historic bacterial infection of the urinary tract was suspected, 16S PCR was performed on 15 urines samples taken in the three years before death. In these samples, positive bands were seen in 13 of the 15 samples. Analysis showed no predominance of one particular genus, meaning it was impossible to confirm a specific historic infection. Additional challenges for interpretation were suboptimal collection and storage of samples in field conditions, and lack of 16S results in similar samples to compare.

The participant could not attend but agreed to include the work in this booklet

Milos Bielcik

Freie Universität Berlin (FU), Berlin

Let us discuss causation in microbiome research

The use of microbial communities to alter host phenotype or ecosystem functions is a promising tool in bio-medicine, biotechnology, agriculture and conservation. Across research disciplines and applications, the progress depends on establishing a causal link between specific community composition and function/phenotype. My contribution stems from previous work of other authors (Lynch et al., Biol Philos 2019) and aims for a critical discussion on the causal link and a brainstorming on how the application of interventionist framework of causation can increase the quality and impact of our research.

The participant could not attend but agreed to include the work in this booklet

Thank you for your participation in our Microbiome Network Meeting

Organization committee (alphabetical order)

Amelie Weber (FU)

Bruno Rocha Cordeiro Costa (Charité)

Carola Susanne Ellner (FU)

Finn Lobnow (HU)

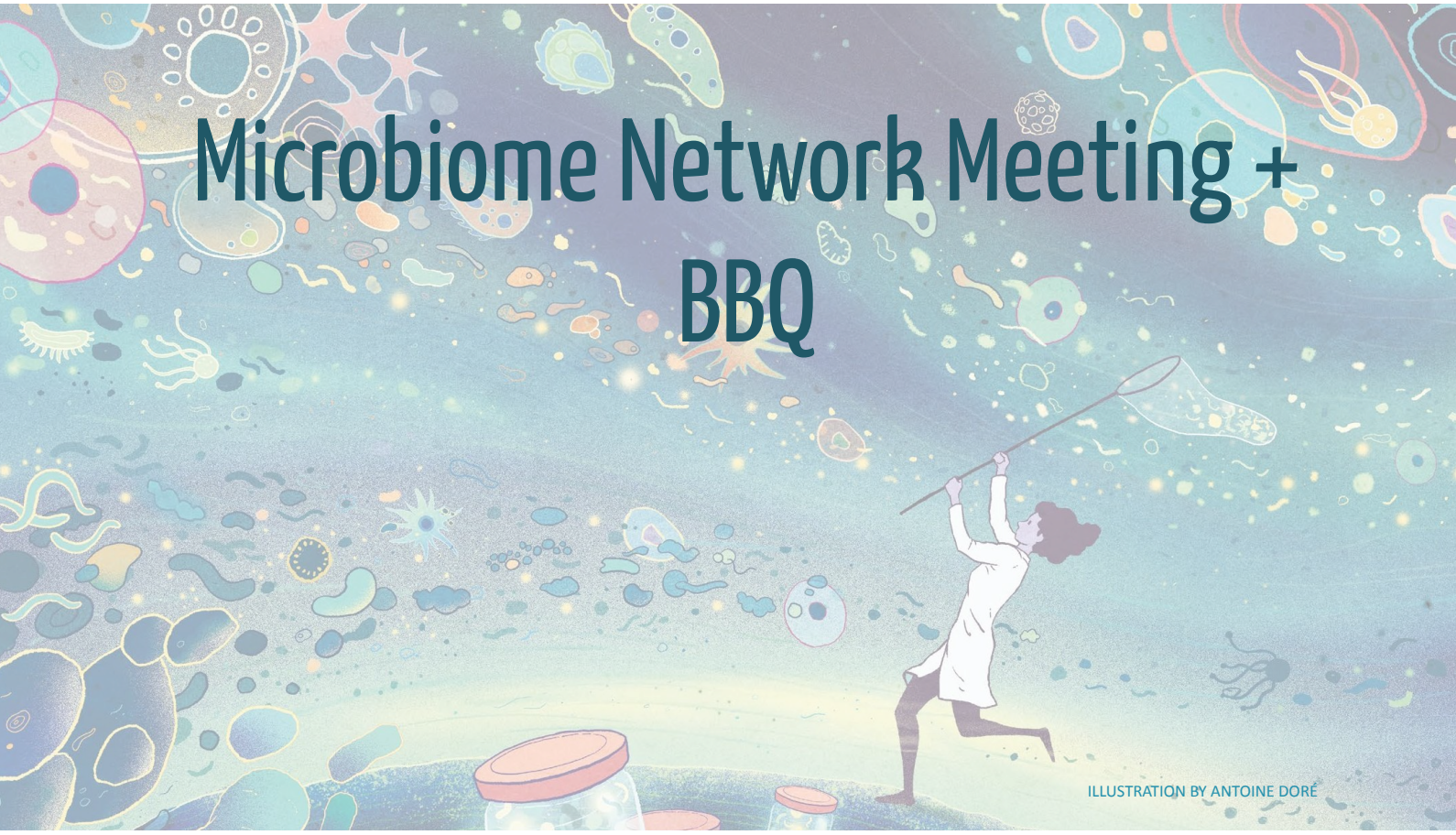
Jürgen Zentek (FU, Food Berlin)

Łukasz Grześkowiak (FU)

Richard Lucius (HU, Food Berlin)

Víctor Hugo Jarquín Díaz (ECRC- MDC/Charité)

Microbiome Network Meeting + BBQ



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