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Institute of Veterinary Bacteriology

Vetsuisse Faculty
Länggassstrasse 122
3012 Bern
Switzerland

Featured image of
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Related article Akarsu et al., 2022

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1 Preface

The Institute of Veterinary Bacteriology is delighted to distribute its Annual Report summarizing the activities and publications of 2022. It features the achievements of our staff, students and collaborators. With respect to collaborative research, I would like to express my gratitude especially to staff from the Institute for Infectious Diseases (IFIK) at the Universty of Bern and the Institute of Virology and Immunology (IVI), with whom we had most fruitful research interactions.

In 2022 we said farewell to four long-serving staff members, namely Tekie Andemicael Weldehiwet, Valentine Jaquier, Susanne Rickli and Sybille Schwendener and we wish them all the best for their new endevoirs. The COVID-19 pandemic affected the year 2022 much less than the previous years and we went back to normal oprating modus. Our teaching portfolio was extended to the module paraclinic days, which aims to familiarize veterinary students with day to day work in paraclinical disciplines.

Our staff published 38 papers with partners and we were able to keep the level of scientific quality. We increased the pool of residents enrolled in the training programme of the European College of Veterinary Microbiology (ECVM) and American Collage of Veterinary Microbiology having three residents and being the largest in Europe. With respect to our environmental footprint, we reviewed our waste management and electricity usage and took measures to reduce our waste amount and to cut our electricity usage.

Altogether, we provided diagnostic services to the university, private practitioners and the Federal Food Safety and Veterinary Office (FSVO) as reference functions and monitoring of antibiotic resistance. I thank all members of the institute, our partners and hope you enjoy reading this annual report.

Bern, in February 2023 Jörg Jores

2 Research Units

2.1 Host-Pathogen Interactions

2.1.1 Genomic Characterization and Antimicrobial Susceptibility of Dromedary-Associated *Staphylococcaceae* from the Horn of Africa

Publication: Hatice Akarsu, Anne Liljander, Mario Younan, Isabelle Brodard, Gudrun Overesch, Ilona Glücks, Fabien Labroussaa, Peter Kuhnert, Vincent Perreten, Stefan Monecke, Jan Felix Drexler, Victor Max Corman, Laurent Falquet, Joerg Jores (2022) Applied and Environmental Microbiology, 88: 01146-22, DOI: 10.1128/aem.01146-22

Collaborators: SIB Swiss Institute of Bioinformatics, Switzerland; International Livestock Research Institute, Nairobi, Kenya; Food and Agriculture Organization of the UN (FAO), UN Cross-Border Hub for NW Syria, Gaziantep, Turkey; Multidisciplinary Center for Infectious Diseases, University of Bern, Bern, Switzerland; Friedrich Schiller University Jena, Institute of Physical Chemistry, Jena, Germany; Institute of Virology, Charité, Universitätsmedizin Berlin, Freie Universität Berlin, Humboldt-Universität, Berlin Institute of Health (BIH), Berlin, Germany; Biochemistry Unit, University of Fribourg, Fribourg, Switzerland.

Abstract: Members of the *Staphylococcaceae* family, particularly those of the genus Staphylococcus, encompass important human and animal pathogens. We collected and characterized *Staphylococcaceae* strains from apparently healthy and diseased camels (n = 84) and cattle (n = 7) in Somalia and Kenya. We phenotypically characterized the strains, including their antimicrobial inhibitory concentrations. Then, we sequenced their genomes using long-read sequencing, closed their genomes, and subsequently compared and mapped their virulence- and resistance-associated gene pools. Genome-based phylogenetics revealed 13 known *Staphylococcaceae* and at least two novel species. East African strains of different species encompassed novel sequence types and phylogenetically distant clades. About one-third of the strains had non-wild-type MICs. They were resistant to at least one of the following antimicrobials: tetracycline, benzylpenicillin, oxacillin, erythromycin, clindamycin, trimethoprim, gentamicin, or streptomycin, encoded by *tet*(K), *blaZ*/bla_{ARL}, *mecA*/*mecA1*, *msrA*/*mphC*, *salA*, *dfrG*, *aacA-aphD*, and *str*, respectively. We identified the first methicillin- and multidrug-resistant camel *S. epidermidis* strain of sequence type (ST) 1136 in East Africa. The pool of virulence-encoding genes was largest in the S. aureus strains, as expected, although other rather commensal strains contained distinct virulence-encoding genes. We identified toxin-antitoxin (TA) systems such as the *hicA*/*hicB* and *abiEii*/*abiEi* families, reported here for the first time for certain species of *Staphylococcaceae*. All strains contained at least one intact prophage sequence, mainly belonging to the *Siphoviridae* family. We pinpointed potential horizontal gene transfers between camel and cattle strains and also across distinct *Staphylococcaceae* clades and species.

2.1.2 Impaired immune response drives age-dependent severity of COVID-19

Publication: Julius Beer, Stefania Crotta, Angele Breithaupt, Annette Ohnemus, Jan Becker, Benedikt Sachs, Lisa Kern, Miriam Llorian, Nadine Ebert, Fabien Labroussaa, Tran Thi Nhu Thao, Bettina Salome Trueeb, Joerg Jores, Volker Thiel, Martin Beer, Jonas Fuchs, Georg Kochs, Andreas Wack, Martin Schwemmle, and Daniel Schnepf (2022) Journal of Experimental Medicine, 219:e20220621 DOI: 10.1084/jem.20220621

Collaborators: Institute of Virology, Medical Center University of Freiburg, Freiburg, Germany; Immunoregulation Laboratory, The Francis Crick Institute, London, UK; Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; Bioinformatics and Biostatistics, The Francis Crick Institute, London, UK; Institute of Virology and Immunology, Bern, Switzerland; Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; Faculty of Medicine, University of Freiburg, Freiburg, Germany.

Abstract: Severity of COVID-19 shows an extraordinary correlation with increasing age.We generated a mouse model for severe COVID-19 and show that the age-dependent disease severity is caused by the disruption of a timely and well-coordinated innate and adaptive immune response due to impaired interferon (IFN) immunity. Aggravated disease in aged mice was characterized by a diminished IFN-γ response and excessive virus replication. Accordingly, adult IFN-γ receptor-deficient mice phenocopied the age-related disease severity, and supplementation of IFN-γ reversed the increased disease susceptibility of aged mice. Further, we show that therapeutic treatment with IFN-λ in adults and a combinatorial treatment with IFN-γ and IFN-λ in aged Ifnar1−/− mice was highly efficient in protecting against severe disease. Our findings provide an explanation for the age-dependent disease severity and clarify the nonredundant antiviral functions of type I, II, and III IFNs during SARS-CoV-2 infection in an age-dependent manner. Our data suggest that highly vulnerable individuals could benefit from immunotherapy combining IFN-γ and IFN-λ.

2.1.3 Serological Diversity of *Dichelobacter nodosus* in German Sheep Flocks

Publication: Monia Budnik, Ann-Kathrin Struck, Julia Storms, Anna Wirth, Jörg Jores, Peter Kuhnert and Ottmar Distl (2022) Animals, 12: 753. DOI: 10.3390/ani12060753

Collaborators: Institute of Animal Breeding and Genetics, University of Veterinary Medicine Hannover (Foundation), 30559 Hannover, Germany

Abstract: Footrot is one of the major causes of lameness in sheep and leads to decreased animal welfare and high economic losses. The causative agent is the Gram-negative anaerobic bacterium *Dichelobacter nodosus*. The prevalence of *D. nodosus* in 207 sheep flocks across Germany was 42.9%. Based on the sequence variation in the type IV fimbrial gene fimA, D. nodosus can be subdivided into ten serogroups (A–I and M). There are commercially available vaccines covering nine serogroups, but the efficacy is low compared to bivalent vaccines. The

aim of this study was to investigate the diversity of serogroups in Germany at the flock and animal levels. In total, we detected at least one serogroup in 819 samples out of 969 D. nodosus-positive samples from 83 flocks using serogroup-specific singleplex PCR for the serogroups A–I. Serogroup A was most prevalent at the animal level, followed by serogroups B, H and C. At the flock level, serogroups A and B had the highest prevalence, each with 64%, but only 40% of flocks had both. The average number of serogroups per animal was 1.42 (range one to five) and, per flock, 3.10 (range one to six). The serogrouping showed within-flock specific clusters but were widely distributed, with 50 different combinations across the flocks. The factors associated with the number of serogroups per animal and single serogroups were the load of D. nodosus, footrot score, sheep breed and flock. Our results indicate that efficient vaccination programs would benefit from tailor-made flock-specific vaccines and regular monitoring of circulating serotypes in the flock to be able to adjust vaccine formulations for nationwide progressive control of footrot in Germany.

2.1.4 Multiparameter flow cytometry assay to analyze the pulmonary T cell profiles in the ovine model of respiratory syncytial virus infection

Publication: Thomas Démoulins, Melanie Brügger, Beatrice Zumkehr, Blandina I Oliveira Esteves, Nicolas Ruggli, Marco P Alves (2022) STAR Protocols, 3(4):101688, DOI: 10.1016/j.xpro.2022.101688

Collaborators: Institute of Virology and Immunology, Bern, Switzerland; Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Abstract: Here, we present a protocol to analyze the T cell profiles of the neonatal ovine lung during respiratory syncytial virus (RSV) infection. The protocol delivers standardized multiparameter flow cytometry (FCM) analysis of $CD4^+$, $CD8^+$, regulatory, and γδ T cells isolated from lung, lymph nodes, and bronchoalveolar lavages (BALs). We detail the preparation of RSV and transtracheal inoculation of newborn lambs. We then describe tissue isolation and preparation of cell suspensions, followed by FCM acquisition to identify different T cell subsets. For complete details on the use and execution of this protocol, please refer to Démoulins et al. (2021).

2.1.5 Seroprevalence of *Mycoplasma hyopneumoniae* in sows fifteen years after implementation of a control programme for enzootic pneumonia in Switzerland

Publication: Nadia Scalisi, Peter Kuhnert, Maria Elena Vargas Amado, Gudrun Overesch, Katharina D.C. Stärk, Nicolas Ruggli, Joerg Jores (2022) Veterinary Microbiology, 270: 109455, DOI: 10.1016/j.vetmic.2022.109455

Collaborators: Department of Geography, University of Zürich, Zürich, Switzerland; Swiss Federal Research Institute WSL, Birmensdorf, Switzerland; Federal Food Safety and Veterinary Office FSVO, Schwarzenburgstrasse 155, 3003 Bern, Switzerland; Institute of Virology and Immunology IVI, Sensemattstrasse 293, 3147 Mittelhäusern, Switzerland;

Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Länggass-Str. 122, PO Box 3001, Bern, Switzerland
Abstract: *Mycoplasma hyopneumoniae* is the etiological agent of enzootic pneumonia (EP), an economically important chronic respiratory disease in pigs. *M. hyopneumoniae* impacts the mucociliary clearance system by disrupting the cilia and modulates the immune response, resulting in intermittent dry non-productive cough. For progressive control of EP in Switzerland, a corresponding programme was fully implemented in 2004. It is based on total depopulation strategies of affected fattening farms as well as partial depopulation in breeding farms. Surveillance of EP status in Switzerland is mainly based on real-time PCR of nasal swabs from coughing animals or suspicious lungs and thereby sporadic cases are still observed every year. In order to obtain information on the seroprevalence, serum samples of 5021 sows from 968 farms collected in 2018 at eight different slaughterhouses were analyzed for the presence of *M. hyopneumoniae*-specific antibodies using a commercial ELISA kit. The overall seroprevalence was low with 0.98% of sows testing positive and these seropositive animals could be allocated to 3.92% of farms tested. Most seropositive farms presented weakly positive singleton reactors and only one farm showed several strongly seropositive animals. In conclusion, the serological status mirrors the successful progressive control of *M. hyopneumoniae* in the Swiss domestic pig population over the years. The current study underlines the added value of serological testing in the surveillance of EP in a country with low prevalence and confirms the sustained benefit of strategic control programmes.

2.1.6 Risk factors associated with the infection of sheep with *Dichelobacter nodosus*

Publication: Julia Storms, Anna Wirth, Danae Vasiliadis, Jörg Jores, Peter Kuhnert & Ottmar Distl (2022) Scientific Reports, 12: 10032, DOI: 10.1038/s41598-022-13933-4
Collaborators: Institute of Animal Breeding and Genetics, University of Veterinary Medicine Hannover, 30559 Hannover, Germany.
Abstract: Ovine footrot is a highly contagious foot disease caused by the gram-negative bacterium *Dichelobacter nodosus* (*D. nodosus*). In a recent report, we showed a prevalence of 42.9% *D. nodosus* positive swabs across Germany. In this follow-up study, we used real-time PCR results for *D. nodosus* and footrot scores of 9297 sheep from 208 flocks and collated these data with survey data on herd and animal characteristics and herd management. The aims of the present study were to investigate herd and animal factors associated with *D. nodosus* infection and footrot scores in individual sheep. Multivariable analyses with generalized mixed models showed that month of recording, breed, herdbook membership, use of antibiotics, and footbaths in the past 3–10 years, signs of footrot in the past 12 months and flock environment of the sheep, modelled as a random farm effect within region, were significant risk factors. Among the 21 different breeds, Romney had the lowest risk of *D. nodosus* infection, while Swifter had the highest risk and German Merino and German White Heath were the next breeds at highest risk of *D. nodosus* infection. The variance between farms in the prevalence of *D. nodosus* was

large and accounted for 84% of the total variance in the mixed model analysis. We conclude that specific and as yet unknown effects influencing *D. nodosus* infections in flocks, as well as breed and weather, are the most important effects on *D. nodosus* infection in sheep, pointing towards the need to establish adequate infection control at farm level.

2.1.7 Genome Engineering of the Fast-Growing *Mycoplasma feriruminatoris* toward a Live Vaccine Chassis

Publication: Vincent Talenton, Vincent Baby, Geraldine Gourgues, Charlotte Mouden, Stephane Claverol, Sanjay Vashee, Alain Blanchard, Fabien Labroussaa, Joerg Jores, Yonathan Arfi, Pascal Sirand-Pugnet, and Carole Lartigue (2022) ACS Synthetic Biology, 11: 1919-30, DOI: 10.1021/acssynbio.2c00062

Collaborators: University of Bordeaux, INRAE, UMR BFP, F-33882 Villenave d'Ornon, France; Département de Biologie, Université de Sherbrooke, J1K 2R1 Sherbrooke, Québec, Canada; INRAE, BIOGECO, F-33610 Cestas, France; Plateforme Proteome, University of Bordeaux, F-33076 Bordeaux, France; J. Craig Venter Institute, Rockville, Maryland 20850, United States

Abstract: Development of a new generation of vaccines is a key challenge for the control of infectious diseases affecting both humans and animals. Synthetic biology methods offer new ways to engineer bacterial chassis that can be used as vectors to present heterologous antigens and train the immune system against pathogens. Here, we describe the construction of a bacterial chassis based on the fast-growing *Mycoplasma feriruminatoris*, and the first steps toward its application as a live vaccine against contagious caprine pleuropneumonia (CCPP). To do so, the *M. feriruminatoris* genome was cloned in yeast, modified by iterative cycles of Cas9-mediated deletion of loci encoding virulence factors, and transplanted back in *Mycoplasma capricolum* subsp. *capricolum* recipient cells to produce the designed *M. feriruminatoris* chassis. Deleted genes encoded the glycerol transport and metabolism systems GtsABCD and GlpOKF and the Mycoplasma Ig binding protein-Mycoplasma Ig protease (MIB-MIP) immunoglobulin cleavage system. Phenotypic assays of the *M. feriruminatoris* chassis confirmed the corresponding loss of H_2O_2 production and IgG cleavage activities, while growth remained unaltered. The resulting mycoplasma chassis was further evaluated as a platform for the expression of heterologous surface proteins. A genome locus encoding an inactivated MIB-MIP system from the CCPP-causative agent *Mycoplasma capricolum* subsp. *capripneumoniae* was grafted in replacement of its homolog at the original locus in the chassis genome. Both heterologous proteins were detected in the resulting strain using proteomics, confirming their expression. This study demonstrates that advanced genome engineering methods are henceforth available for the fast-growing *M. feriruminatoris*, facilitating the development of novel vaccines, in particular against major mycoplasma diseases.

2.1.8 Enhanced fitness of SARS-CoV-2 variant of concern Alpha but not Beta

Publication: Lorenz Ulrich, Nico Joel Halwe, Adriano Taddeo, Nadine Ebert, Jacob Schön, Christelle Devisme, Bettina Salome Trüeb, Bernd Hoffmann, Manon Wider, Xiaoyu Fan, Meriem Bekliz, Manel Essaidi-Laziosi, Marie Luisa Schmidt, Daniela Niemeyer, Victor Max Corman, Anna Kraft, Aurélie Godel, Laura Laloli, Jenna N. Kelly, Brenda M. Calderon, Angele Breithaupt, Claudia Wylezich, Inês Berenguer Veiga, Mitra Gultom, Sarah Osman, Bin Zhou, Kenneth Adea, Benjamin Meyer, Christiane S. Eberhardt, Lisa Thomann, Monika Gsell, Fabien Labroussaa, Jörg Jores, Artur Summerfield, Christian Drosten, Isabella Anne Eckerle, David E. Wentworth, Ronald Dijkman, Donata Hoffmann, Volker Thiel, Martin Beer & Charaf Benarafa (2022) Nature, 602: 307-13, DOI: 10.1038/s41586-021-04342-0

Collaborators: Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; Institute of Virology and Immunology, Mittelhäusern, Switzerland; Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Institute for Infectious Diseases, University of Bern, Bern, Switzerland; CDC COVID-19 Emergency Response, Centers for Disease Control and Prevention, Atlanta, GA, USA; Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland; Charité–Universitätsmedizin Berlin, Institute of Virology, Berlin, Germany; German Centre for Infection Research (DZIF), Berlin, Germany; Graduate School for Biomedical Science, University of Bern, Bern, Switzerland; Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; Centre for Vaccinology, Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland; Division of General Paediatrics, Department of Woman, Child and Adolescent Medicine, Faculty of Medicine, University of Geneva, Geneva, Switzerland; Center for Vaccinology, Geneva University Hospitals, Geneva, Switzerland; Division of Infectious Disease, Geneva University Hospitals, Geneva, Switzerland; Division of Laboratory Medicine, Laboratory of Virology, Geneva University Hospitals, Geneva, Switzerland.

Abstract: Emerging variants of concern (VOCs) are driving the COVID-19 pandemic. Experimental assessments of replication and transmission of major VOCs and progenitors are needed to understand the mechanisms of replication and transmission of VOCs3. Here we show that the spike protein (S) from Alpha (also known as B.1.1.7) and Beta (B.1.351) VOCs had a greater affinity towards the human angiotensin-converting enzyme 2 (ACE2) receptor than that of the progenitor variant S(D614G) in vitro. Progenitor variant virus expressing S(D614G) (wt-S^{614G}) and the Alpha variant showed similar replication kinetics in human nasal airway epithelial cultures, whereas the Beta variant was outcompeted by both. In vivo, competition experiments showed a clear fitness advantage of Alpha over wt-S^{614G} in ferrets and two mouse models—the substitutions in S were major drivers of the fitness advantage. In hamsters, which support high viral replication levels, Alpha and wt-S^{614G} showed similar fitness. By contrast, Beta was outcompeted by Alpha and wt-S^{614G} in hamsters and in mice expressing human ACE2.

Our study highlights the importance of using multiple models to characterize fitness of VOCs and demonstrates that Alpha is adapted for replication in the upper respiratory tract and shows enhanced transmission in vivo in restrictive models, whereas Beta does not overcome Alpha or wt-S^{614G} in naive animals.

2.1.9 The XadA trimeric autotransporter adhesins in *Xylella fastidiosa* differentially contribute to cell aggregation, biofilm formation, insect transmission and virulence to plants

Publication: Oseias R. Feitosa-Junior, Ana Paula S. Souza, Paulo A. Zaini, Claudia Baccari, Michael Ionescu, Paulo M. Pierry, Guillermo Uceda-Campos, Fabien Labroussaa, Rodrigo P.P. Almeida, Steven E. Lindow and Aline M. da Silva (2022) Molecular Plant Microbe Interactions, 35(9):857-866, DOI: 10.1094/MPMI-05-22-0108-R

Collaborators: Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil; Department of Plant Sciences, University of California Davis, U.S.A; Department of Plant and Microbial Biology, University of California Berkeley, U.S.A; Department of Environmental Science, Policy and Management, University of California Berkeley, U.S.A.

Abstract: Surface adhesion strategies are widely employed by bacterial pathogens during establishment and systemic spread in their host. A variety of cell-surface appendages such as pili, fimbriae, and afimbrial adhesins are involved in these processes. The phytopathogen *Xylella fastidiosa* employs several of these structures for efficient colonization of its insect and plant hosts. Among the adhesins encoded in the *X. fastidiosa* genome, three afimbrial adhesins, XadA1, Hsf/XadA2, and XadA3, are predicted to be trimeric autotransporters with a C-terminal YadA-anchor membrane domain. We analyzed the individual contributions of XadA1, XadA2, and XadA3 to various cellular behaviors both in vitro and in vivo. Using isogenic *X. fastidiosa* mutants, we found that cell-cell aggregation and biofilm formation were severely impaired in the absence of XadA3. No significant reduction of cell-surface attachment was found with any mutant under flow conditions. Acquisition by insect vectors and transmission to grapevines were reduced in the XadA3 deletion mutant. While the XadA3 mutant was hypervirulent in grapevines, XadA1 or XadA2 deletion mutants conferred lower disease severity than the wild-type strain. This insight of the importance of these adhesive proteins and their individual contributions to different aspects of *X. fastidiosa* biology should guide new approaches to reduce pathogen transmission and disease development.

2.1.10 Investigating the biocontrol potential of the natural microbiota of the apple blossom

Publication: Anya Schnyder, Leo Eberl and Kirsty Agnoli (2022) Microorganisms, 10(12):2480, DOI: 10.3390/microorganisms10122480

Collaborators: Department of Microbiology, Institute of Plant and Microbial Biology, University of Zürich, Switzerland.

Abstract: Erwinia amylovora, the causative agent of fire blight, leads to important economic losses of apple and pear crops worldwide. This study aimed to investigate the potential of the resident microbiota of the apple blossom in combatting plant disease-causing organisms, with a focus on controlling fire blight. We obtained 538 isolates from sites around Canton Zurich, which we tested for activity against *Pectobacterium carotovorum* and *E. amylovora*. We also evaluated the isolates' activity against oomycete and fungal pathogens. Nine isolates showed activity against *P. carotovorum*, and eight of these against *E. amylovora*. Furthermore, 117 showed antifungal, and 161 anti-oomycete, activity. We assigned genera and in some cases species to 238 of the isolates by sequencing their 16S RNA-encoding gene. Five strains showed activity against all pathogens and were tested in a detached apple model for anti-*E. amylovora* activity. Of these five strains, two were able to antagonize *E. amylovora*, namely *Bacillus velezensis* #124 and *Pantoea agglomerans* #378. We sequenced the *P. agglomerans* #378 genome and analyzed it for secondary metabolite clusters using antiSMASH, revealing the presence of a putative bacteriocin cluster. We also showed that *B. velezensis* #124 exhibits strong activity against three different fungi and two oomycetes *in vitro*, suggesting a broader capacity for biocontrol. Our results showcase the protective potential of the natural apple blossom microbiota. We isolated two candidate biocontrol strains from apple blossoms, suggesting that they might persist at the most common entry point for the causative agent of fire blight. Furthermore, they are probably already part of the human diet, suggesting they might be safe for consumption, and thus are promising candidates for biocontrol applications.

2.1.11 *Wielerella bovis* gen. nov., sp. nov. a member of the family *Neisseriaceae* associated with bovine endocarditis

Publication: Peter Kuhnert, Isabelle Brodard, Sabine Bock, Andrew Hemphill, Hatice Akarsu, Andreas Engelhardt, Peter Kutzer (2022) Int.J.Syst.Evol.Microbiol. 72:005387, DOI: 10.1099/ijsem.0.005387

Collaborators: Landeslabor Berlin-Brandenburg, Frankfurt (Oder), Germany.

Abstract: Seven bacterial strains isolated from bovine endocarditis in six animals from different geographic regions were investigated in a polyphasic taxonomic approach. Phylogenetic analysis based on 16S rRNA gene sequences placed all seven isolates on a distinct, monophyletic cluster in the family *Neisseriaceae* with closest similarity to type strains of *Alysiella filiformis* (97.06%) and *Kingella kingae* (96.34%). Whole genome sequence (WGS) analysis of isolates confirmed their species status, with an average nucleotide identity (ANI) >96% between isolates and <80% to other type species of genera of *Neisseriaceae* while digital DNA-DNA hybridization (dDDH) values were >80% and <18%, respectively. The DNA G+C content was 42.5-43.0 mol%. WGS based phylogeny showed the isolates being monophyletic and separated from established genera thereby forming a new genus within the family *Neisseriaceae*. Similarly, analysis of MALDI-TOF MS reference spectra clustered the isolates close together and clearly separated from other genera, making this the method of choice for

identification. Biochemical markers based on classical as well as commercial identification schemes allowed separation from closely related *Neisseriaceae* genera, even though the new taxon is biochemically not very active. Major fatty acids are $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$. The major quinone is ubiquinone Q-8. In the polar lipid profile, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phospholipid were predominant. We propose the novel genus *Wielerella* with the type species *Wielerella bovis* gen. nov., sp. nov.. The type strain is CCUG 44465^T (= DSM 113289^T = JF 2483^T) isolated postmortem from a cow with endocarditis in Switzerland.

2.1.12 Detection of Testudinid alphaherpesvirus, *Chlamydia* spp., *Mycoplasma* spp., and *Salmonella* spp. in free-ranging and rescued Italian *Testudo hermanni hermanni*

Publication: Maria Luisa Marenzoni, Valentina Stefanetti, Emilia Del Rossi, Alessia Zicavo, Stefania Scuota, Francesco Carlo Origgi, Gianluca Deli, Claudia Corti, Massimo Trabalza Marinucci, Oliviero Olivieri (2022) Veterinaria Italiana 58:25-34, DOI: 10.12834/VetIt.1915.13833.1

Collaborators: University of Perugia, Italy; Istituto Zooprofilattico Umbria e Marche "Togo Rosati", Italy; University of Florence, Italy.

Abstract: Testudo hermanni is included as near-threatened in the Red List of the International Union for Conservation of Nature, while *T. hermanni hermanni* is considered endangered in the Italian Red List. Appropriate management of smuggled or seized wild individuals is recommended before their reintroduction into the wild. Accordingly, a health monitoring study was carried out. During 2014-2016, 133 oral swabs and 121 cloacal swabs were collected from a total of approximately 180 free-ranging and rescued *T. hermanni hermanni* from eight different Italian regions to investigate the presence of DNA of Testudinid alphaherpesvirus (TeAHV), *Chlamydia* spp. and *Mycoplasma* spp. in the oral cavity, and *Salmonella* spp. isolates in the cloaca. *Mycoplasma* spp. was detected in 52 out of 87 (59.77%) of rescued and in 1 out of 46 free-ranging (2.17%) individuals; 33 out of 53 (62.26%) *Mycoplasma* spp. positive samples were typed as *M. agassizii* by PCR. *Salmonella* spp. was isolated from 45 out of 121 (37.19%) cloacal swabs, typed into 14 serovars, and characterized for complete antimicrobial susceptibility. A significantly different distribution of *Salmonella* spp. isolates was found in 2016 in comparison with 2014 and 2015, without any difference between free-ranging and rescued tortoises. All the tested tortoises were negative for TeAHV and *Chlamydia* spp. These results are considered a baseline information critical to monitor the dynamics of these microorganisms in free-ranging and rescued populations of *T. h. hermanni*, and to correctly approach the management of rescued animals and possible relocation programs.

2.1.13 Substrate recognition and cryo-EM structure of the ribosome-bound TAC toxin

of *Mycobacterium tuberculosis*

Publication: Moise Mansour, Emmanuel Giudice, Xibing Xu, Hatice Akarsu, Patricia Bordes, Valérie Guillet, Donna-Joe Bigot, Nawel Slama, Gaetano D'urso, Sophie Chat, Peter Redder, Laurent Falquet, Lionel Mourey, Reynald Gillet & Pierre Genevaux (2022) Nat Commun 13:2641. DOI: 10.1038/s41467-022-30373-w.

Collaborators: Laboratoire de Microbiologie et de Genetique Moleculaires, Centre de Biologie Integrative (CBI), Universite de Toulouse, CNRS, UPS, Toulouse, France; Institut de Genetique et Developpement de Rennes (IGDR), UMR6290, Universite de Rennes, CNRS, Rennes, France; Department of Biology, University of Fribourg & Swiss Institute of Bioinformatics, Fribourg, Switzerland; Institut de Pharmacologie et de Biologie Structurale, IPBS, Universite de Toulouse, CNRS, UPS, Toulouse, France

Abstract: Toxins of toxin-antitoxin systems use diverse mechanisms to control bacterial growth. Here, we focus on the deleterious toxin of the atypical tripartite toxin-antitoxin-chaperone (TAC) system of *Mycobacterium tuberculosis*, whose inhibition requires the concerted action of the antitoxin and its dedicated SecB-like chaperone. We show that the TAC toxin is a bona fide ribonuclease and identify exact cleavage sites in mRNA targets on a transcriptome-wide scale in vivo. mRNA cleavage by the toxin occurs after the second nucleotide of the ribosomal A-site codon during translation, with a strong preference for CCA codons *in vivo*. Finally, we report the cryo-EM structure of the ribosome-bound TAC toxin in the presence of native *M. tuberculosis cspA* mRNA, revealing the specific mechanism by which the TAC toxin interacts with the ribosome and the tRNA in the P-site to cleave its mRNA target.

2.2 Molecular and Bacterial Epidemiology and Infectious Diseases

2.2.1 Whole-genome sequences of antibiotic-resistant *Trueperella pyogenes* isolates from surgical site infections in dairy cows in Switzerland

Publication: Emma Marchionatti, Vincent Perreten (2022) Microbiol Resour Announc. 11(12):e0086522, DOI: 10.1128/mra.00865-22

Collaborators: Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Abstract: The complete genome sequence of four *Trueperella pyogenes* isolated from cattle surgical site infections in Switzerland was determined using hybrid assembly of Oxford Nanopore and Illumina reads. Genes conferring resistance to tetracyclines [*tet*(W)], sulfonamides (*sul1*), chloramphenicol (*cmx*), streptomycin/spectinomycin (*aadA1*), and quaternary ammonium compounds (*qacEΔ1*) were identified on different chromosomal elements.

2.2.2 *mcr-1* colistin resistance gene sharing between *Escherichia coli* from cohabiting

dogs and humans, Lisbon, Portugal, 2018 to 2020

Publication: Juliana Menezes, Joana Moreira da Silva, Sian-Marie Frosini, Anette Loeffler, Scott Weese, Vincent Perreten, Stefan Schwarz, Luís Telo da Gama, Andreia Jesus Amaral, Constança Pomba (2022) Euro Surveill. 27(44):2101144, DOI: 10.2807/1560-7917.ES.2022.27.44.2101144

Collaborators: Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal; Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal; Royal Veterinary College, Hertfordshire, United Kingdom; Ontario Veterinary College, Guelph, Ontario, Canada; Centre for Infection Medicine, Department of Veterinary Medicine, Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany.

Abstract: The emergence of colistin resistance is a One Health antimicrobial resistance challenge worldwide. The close contact between companion animals and humans creates opportunities for transmission and dissemination of colistin-resistant bacteria. The study aimed to detect potential animal reservoirs of colistin-resistant *Escherichia coli* and investigate the possible sharing of these bacteria between dogs, cats and their cohabiting humans in the community in Lisbon, Portugal. A prospective longitudinal study was performed from 2018 to 2020. Faecal samples from dogs and cats either healthy or diagnosed with a skin and soft tissue or urinary tract infection, and their cohabiting humans were screened for the presence of colistin-resistant *E. coli.* All isolates were tested by broth microdilution against colistin and 12 other antimicrobials. Colistin-resistant isolates were screened for 30 resistance genes, including plasmid-mediated colistin resistance genes (*mcr-1* to *mcr-9*), and typed by multilocus sequence typing. Genetic relatedness between animal and human isolates was analysed by whole genome sequencing. Colistin-resistant *E. coli* strains harbouring the *mcr-1* gene were recovered from faecal samples of companion animals (8/102; 7.8%) and humans (4/125; 3.2%). No difference between control and infection group was detected. Indistinguishable multidrug-resistant *E. coli* ST744 strains harbouring the *mcr-1* gene were found in humans and their dogs in two households. The identification of identical *E. coli* strains containing the plasmid-mediated *mcr-1* gene in companion animals and humans in daily close contact is of concern. These results demonstrated the importance of the animal-human unit as possible disseminators of clinically important resistance genes in the community setting.

2.2.3 Intra- and interspecies spread of a novel conjugative multidrug resistance IncC plasmid coharboring *bla*$_{\text{OXA-181}}$ and *arm*A in a cystic fibrosis patient

Publication: Javier E. Fernandez, Helena M.B. Seth-Smith, Patrice Nordmann, Adrian Egli, Andrea Endimiani, Vincent Perreten (2022) Microbiol Spectr. 10(5):e0312122, DOI: 10.1128/spectrum.03121-22

Collaborators: Clinical Bacteriology and Mycology, University Hospital Basel, Basel, Switzerland; Applied Microbiology Research, Department of Biomedicine, University of

Basel, Basel, Switzerland; Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland; Institute for Infectious Diseases (IFIK), University of Bern, Bern, Switzerland.

Abstract: A novel multidrug resistance conjugative 177,859-bp IncC plasmid pJEF1-OXA-181 coharboring the carbapenemase-coding *bla*$_{OXA181}$ and the aminoglycoside resistance 16S rRNA methyltransferase-coding *armA* genes was detected in two unrelated Escherichia coli gut isolates of ST196 and ST648, as well as two ST35 Klebsiella pneumoniae gut and sputum isolates of a cystic fibrosis patient. The *armA* gene was located within the antimicrobial resistance island ARI-A and the *bla*$_{OXA181}$ gene, which was preceded by IS*903* and IS*Ecp1Δ* was inserted within the transfer genes region without affecting conjugation ability. Comparative plasmid analysis with other related IncC plasmids showed the presence of *bla*$_{OXA181}$, as well as its integration site, are thus far unique for these types of plasmids. This study illustrates the potential of a promiscuous multidrug resistance plasmid to acquire antibiotic resistance genes and to disseminate in the gut of the same host.

2.2.4 Molecular analysis of OXA-48-producing *Escherichia coli* in Switzerland from 2019 to 2020

Publication: Jacqueline Findlay, Vincent Perreten, Laurent Poirel, Patrice Nordmann (2022) Eur J Clin Microbiol Infect Dis. 41(11):1355-1360, DOI: 10.1007/s10096-022-04493-6

Collaborators: Medical and Molecular Microbiology, Department of Medicine, Faculty of Science and Medicine, University of Fribourg, Chemin du Musée 18, Fribourg, Switzerland; Medical and Molecular Microbiology, Department of Medicine, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland; Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland; INSERM European Unit (IAME, France); Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland.

Abstract: OXA-48-type ß-lactamases are the most prevalent carbapenemase-type in Enterobacterales in Switzerland, predominantly found in *Escherichia coli* and *Klebsiella pneumoniae*. Bacteria-producing OXA-48-type enzymes are endemic in some parts of the world, including Europe and North Africa, and are a frequent cause of nosocomial infections. Despite the emergence of numerous OXA-48-type variants, the original variant, OXA-48, remains the most prevalent in *E. coli*. This study describes the epidemiology of OXA-48-producing *E. coli* isolates submitted to the Swiss National Reference Center for Emerging Antibiotic Resistance (NARA) between January 2019 and December 2020.

2.2.5 Genome stability during serial subculturing in hyperepidemic multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*

Publication: Aline I Moser, Edgar I Campos-Madueno, Vincent Perreten, Andrea Endimiani (2022) J Glob Antimicrob Resist. 31:152-161, DOI: 10.1016/j.jgar.2022.08.014

Collaborators: Institute for Infectious Diseases, University of Bern, Bern, Switzerland

Abstract: Core-genome single nucleotide variant (cgSNV) analysis represents a powerful tool for epidemiological investigations of multidrug-resistant (MDR) bacteria. However, cgSNV thresholds to confirm whether isolates are the same clone are not formally defined. We implemented hybrid whole-genome sequencing to study the genomic changes of four MDR isolates belonging to hyperepidemic sequence types (STs) during 20 propagation steps (T20) on MacConkey and CHROMID$^{(R)}$ ESBL plates. The following strains were analyzed: *Klebsiella pneumoniae* AE-2247421 (OXA-48/NDM-1-producing, ST101), *K. pneumoniae* MCL-2017-2 (CTX-M-15-producing, ST307), *Escherichia coli* Ec-042 (OXA-181-producing, ST410), and *E. coli* Ec-050 (NDM-5-producing, ST167). The genome assembly at T5 and T20 was compared to that at time point zero (T0) and to two reference genomes. At T20, AE-2247421 lost the IncL *bla*$_{\text{OXA-48}}$-carrying plasmid when grown on CHROMID$^{(R)}$ ESBL plates, while a large fragment encompassing bla$_{\text{NDM-1}}$ was lost from its IncC plasmid when grown on both plates. In contrast, no structural changes were noted for the other three strains. Regarding the cgSNVs, the following results were obtained at T5 and T20 (ranges considering the different agar plates and reference genomes): AE-2247421 (1-8 and 2-12 cgSNVs), MCL-2017-2 (both 1-2 cgSNVs), Ec-042 (both 0 cgSNVs), and Ec-050 (0-6 and 0-9 cgSNVs). We showed that structural changes and accumulation of cgSNVs can occur in few propagation steps under laboratory conditions. These changes might also arise in the clinical context in a short time, especially under antibiotics treatment. This phenomenon should be carefully considered because it might affect the final interpretation of epidemiological genomic analyses.

2.2.6 Clonal dissemination of MDR *Pasteurella multocida* ST79 in a small Swiss veal calf farm with high use of antibiotics

Publication: Jens Becker, Javier E Fernandez, Alexandra Rossano, Mireille Meylan, Vincent Perreten (2022) J Antimicrob Chemother. 77(10):2886-2888, DOI: 10.1093/jac/dkac270

Collaborators: Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Bern, Switzerland

Abstract: In the framework of a large field study where prevalence of and antimicrobial resistance in *P. multocida* in calves was investigated in 38 Swiss farms, we observed that 20 isolates exhibited an MDR profile. The isolates belonged to ST79 and contained an adenine to guanine substitution in each of the six copies of the 23S rRNA gene at position 2058, which confers resistance to macrolides in P. multocida. They also carried the tetracycline efflux gene tet(H), the sulphonamide-resistant dihydropteroate synthase gene sul2, the spectinomycin/streptomycin adenylyltransferase gene *aadA31*, the streptomycin phosphotransferase genes *strA* and *strB*, and the kanamycin/neomycin phosphotransferase gene *aph(3')-Ia*. The genes were located on a chromosomal integrative and conjugative element (ICE). The isolates also contained an *ftsI* gene mutation, generating an alanine to serine substitution at amino acid position in PBP3 associated with β-lactam resistance. Clonal dissemination of *P. multocida* ST79 was observed throughout a period that was distinctly longer

than the lifespan of a calf, indicating that *P. multocida* ST79 was maintained within the herd by circulating among calves that were present on the farm at the same time.

2.2.7 Associations of antimicrobial use with antimicrobial susceptibility at the calf level in bacteria isolated from the respiratory and digestive tracts of veal calves before slaughter

Publication: Jens Becker, Vincent Perreten, Gertraud Schüpbach-Regula, Dimitri Stucki, Adrian Steiner, Mireille Meylan (2022) J Antimicrob Chemother. 2022 Sep 30;77(10):2859-2866, DOI: 10.1093/jac/dkac246

Collaborators: Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Switzerland

Abstract: Antimicrobial drugs are frequently administered in veal calves, but investigations on associations with antimicrobial susceptibility of bacteria are scarce and convey partly contradictory findings. The aim of this study was to investigate associations of antimicrobial use (AMU) during the fattening period with antimicrobial susceptibility shortly before slaughter. Detailed treatment data of 1905 veal calves from 38 farms were collected prospectively during monthly farm visits for 1 year (n = 1864 treatments, n = 535 visits); 1582 *Escherichia coli*, 1059 *Pasteurella multocida* and 315 *Mannheimia haemolytica* were isolated from rectal and nasopharyngeal swabs collected before slaughter and subjected to antimicrobial susceptibility testing by microdilution. Associations of antimicrobial treatments with resistant isolates were investigated at the calf level. Associations of AMU with antimicrobial resistance were observed using generalized linear models. For *E. coli*, the odds of being resistant were increased with increased AMU (OR 1.36 when number of treatments >1, P = 0.066). Use of tetracyclines was associated with resistance to tetracycline (OR 1.86, P < 0.001) and use of penicillins was associated with resistance to ampicillin (OR 1.66, P = 0.014). No significant associations were observed for P. multocida (use of aminoglycosides: OR 3.66 for resistance to spectinomycin, P = 0.074). For M. haemolytica, the odds of being resistant were increased with increased AMU (OR 4.63, P < 0.001), and use of tetracyclines was associated with resistance to tetracycline (OR 6.49, P < 0.001). Occurrence of resistant bacteria shortly before slaughter was associated with AMU in veal calves. Prudent and appropriate use may contribute to limit the selection of resistant bacteria on veal farms.

2.2.8 Prevalence and whole genome-based phylogenetic, virulence and antibiotic resistance characteristics of nasal *Staphylococcus aureus* in healthy Swiss horses

Publication: Joel I. Hurni, Sarah Kaiser-Thom, Vinzenz Gerber, Jennifer E. Keller, Alexandra Collaud, Javier E. Fernandez , Sybille Schwendener, Vincent Perreten (2022) Schweiz Arch Tierheilkd. 164(7):499-512, DOI: 10.17236/sat00360

Collaborators: Swiss Institute of Equine Medicine (ISME), Vetsuisse Faculty, University of Bern, and Agroscope, Switzerland.

Abstract: A total of 100 nasal swabs were collected from healthy horses in Switzerland between January 2020 and August 2020. The samples were taken from horses at 40 different stables in 12 different cantons and screened for both methicillin-resistant (MRSA) and methicillin-susceptible *S. aureus* (MSSA) using selective agar plates. S. aureus were tested for antibiotic susceptibility by measurement of the minimal inhibitory concentration (MIC) and for virulence factors, antibiotic resistance genes and phylogenetic characteristics using whole genome sequence analysis. Ten horses were found to be positive (10 %, CI: 95 %, 0,0552 - 0,1744) for *S. aureus*, and four of them harboured MRSA (4 %, CI: 95 %, CI: 1,5 % - 9 %). The MRSA were detected in horses from three different stables in the same region of one canton and MSSA were detected in horses from five different cantons. All the MRSA isolates were genetically related (ST398-t011-IVa), while the MSSA were diverse (ST1-t127/t398/t1508, ST816-t1294, ST133-t1403, ST30-t012). MRSA showed resistance to penicillin (*blaZ*), cefoxitin (*mecA*), trimethoprim (*dfrK*), gentamicin, kanamycin (*aac(6')-Ie–aph(2'')-Ia*), and tetracycline (*tet*(M)). MSSA were resistant to either none or one of the antibiotics tested like penicillin (*blaZ*) and erythromycin (*erm*(T)). Virulence genes were more abundant in MSSA than in MRSA. This study provides first insight into the prevalence and type of *S. aureus* in healthy Swiss horses and reveals a source of strains, which may cause infections in both horses and humans.

2.2.9 Antimicrobial susceptibility in *E. coli* and *Pasteurellaceae* at the beginning and at the end of the fattening process in veal calves: Comparing 'outdoor veal calf' and conventional operations

Publication: Jens Becker, Vincent Perreten, Adrian Steiner, Dimitri Stuck, Gertraud Schüpbach-Regula, Alexandra Collaud, Alexandra Rossano, Dominik Wüthrich, Anna Muff-Hausherr, Mireille Meylan (2022) Vet Microbiol. 269:109419, DOI: 10.1016/j.vetmic.2022.109419

Collaborators: Clinic for Ruminants, Vetsuisse-Faculty, University of Bern, Switzerland; Veterinary Public Health Institute, Vetsuisse-Faculty, University of Bern, Switzerland

Abstract: Animal husbandry requires practical measures to limit antimicrobial resistance (AMR). Therefore, a novel management and housing concept for veal calf fattening was implemented on 19 intervention farms (IF) and evaluated regarding its effects on AMR in *Escherichia* (*E.*) *coli*, *Pasteurella* (*P.*) *multocida* and *Mannheimia* (*M.*) *haemolytica* in comparison with 19 conventional control farms (CF). Treatment intensity (-80%) and mortality (-50%) were significantly lower in IF than in CF, however, production parameters did not differ significantly between groups. Rectal and nasopharyngeal swabs were taken at the beginning and the end of the fattening period. Susceptibility testing by determination of the minimum inhibitory concentration was performed on 5420 isolates. The presence of AMR was described as prevalence of resistant isolates (%), by calculating the Antimicrobial Resistance Index (ARI: number of resistance of one isolate to single drugs/total number of drugs tested), by the occurrence of pansusceptible isolates (susceptible to all tested drugs, ARI=0), and by

calculating the prevalence of multidrug (≥3) resistant isolates (MDR). Before slaughter, odds for carrying pansusceptible *E. coli* were higher in IF than in CF (+65%, p=0.022), whereas ARI was lower (-16%, p=0.003), and MDR isolates were less prevalent (-65%, p=0.001). For P. multocida, odds for carrying pansusceptible isolates were higher in IF before slaughter compared to CF (+990%, p=0.009). No differences between IF and CF were seen regarding the prevalence of pansuceptible *M. haemolytica*. These findings indicate that easy-to-implement measures to improve calf management can lead to a limitation of AMR in Swiss veal fattening farms.

2.2.10 The *bla* and *mec* families of β-lactam resistance genes in the genera *Macrococcus, Mammaliicoccus* and *Staphylococcus*: an in-depth analysis with emphasis on *Macrococcus*

Publication: Sybille Schwendener, Vincent Perreten (2022) J Antimicrob Chemother. 77(7):1796-1827, DOI: 10.1093/jac/dkac107

Abstract: β-Lactamases (Bla) and low-affinity penicillin-binding proteins (PBP2A) are responsible for β-lactam resistance in the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*. These resistance mechanisms are in most species acquired through mobile genetic elements that carry a *blaZ*-like β-lactamase gene for penicillin resistance and/or a *mec* gene (*mecA*, *mecB*, *mecC*,*mecD*) encoding a PBP2A for resistance to virtually all classes of β-lactams. The *mecA* and *mecC* genes can be acquired through staphylococcal cassette chromosome *mec* (SCC*mec*) elements in *Staphylococcus* and *Mammaliicoccus*. The *mecB* and *mecD* genes are found in *Macrococcus* on SCC*mec* elements, as well as on unrelated *mecD*-carrying *Macrococcus* resistance islands (McRI$_{mecD}$) and large *mecB*-carrying plasmids. This review provides a phylogenetic overview of *Macrococcus*, *Mammaliicoccus* and *Staphylococcus* species and an in-depth analysis of the genetic structures carrying *bla* and *mec* genes in these genera. Native *bla* genes were detected in species belonging to the novobiocin-resistant *Staphylococcus saprophyticus* group and *Mammaliicoccus*. The evolutionary relatedness between *Macrococcus* and *Mammaliicoccus* is illustrated on the basis of a similar set of intrinsic PBPs, especially, the presence of a second class A PBP. The review further focuses on macrococcal elements carrying *mecB* and *mecD*, and compares them with structures present in *Staphylococcus* and *Mammaliicoccus*. It also discusses the different recombinases (*ccr* of SCC*mec*) and integrases (*int* of McRI) that contribute to the mobility of methicillin resistance genes, revealing *Macrococcus* as an important source for mobilization of antibiotic resistance genes within the family of *Staphylococcaceae*.

2.2.11 Distribution, genetic heterogeneity, and antimicrobial susceptibility of *Brachyspira pilosicoli* in Swiss pig herds

Publication: Mirjam Arnold, Sarah Schmitt, Alexandra Collaud, Alexandra Rossano, Ella Hübschke, Friederike Zeeh, Heiko Nathues, Vincent Perreten (2022) Vet Microbiol. 269:109421, DOI: 10.1016/j.vetmic.2022.109421

Collaborators: Clinic for Swine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Section of Veterinary Bacteriology, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Abstract: Brachyspira (*B.*) *pilosicoli* is a bacterium causing porcine intestinal spirochaetosis, a disease characterized by diarrhoea and depressed growth rates especially in nursery and fattening pigs. Knowledge of the epidemiology and antimicrobial susceptibility of this pathogen is limited. Therefore, the aim of this study was to analyse the distribution, genetic heterogeneity, and antimicrobial susceptibility of *B. pilosicoli* field isolates from Swiss pig farms. Faecal swabs of 693 animals originating from 156 herds were analysed for the presence of *Brachyspira* spp. using culture and polymerase chain reaction identification. Further characterisation was performed using multilocus sequence typing (MLST) and broth dilution antimicrobial susceptibility testing. With 52.6% positive herds, *B. pilosicoli* could be frequently isolated from herds with animals suffering from diarrhoea. In herds with animals without clinical signs of diarrhoea, detection was significantly less frequent with only 10.5% positive herds (p 0.001). Among 80 isolates used for typing, genetic heterogeneity was observed with 44 different sequence types (ST) which often differed from herd to herd. No predominant ST was observed. More than 73.0% of the 41 *B. pilosicoli* isolates analysed, showed minimal inhibitory concentration values above the wild type cut-off values for lincomycin, tylvalosin and/ or tylosin. For tiamulin, valnemulin and doxycycline, this was the case in 48.8%, 43.9% and 36.6%, respectively. In conclusion, a diverse population of *B. pilosicoli* exhibited decreased susceptibility to antimicrobials used against *Brachyspira* infections. Monitoring of resistance in *Brachyspira* spp. is highly recommended to support targeted use of antimicrobials in pigs.

2.2.12 Prevalence and WGS-based characteristics of *Staphylococcus aureus* in the nasal mucosa and pastern of horses with equine pastern dermatitis

Publication: Sarah Kaiser-Thom, Vinzenz Gerber, Alexandra Collaud, Joel Hurni, Vincent Perreten (2022) BMC Vet Res. 18(1):79, DOI: 10.1186/s12917-021-03053-y

Collaborators: Department of Clinical Veterinary Medicine, Vetsuisse Faculty, Swiss Institute of Equine Medicine (ISME), University of Bern, and Agroscope, Bern, Switzerland

Abstract: Many contributing factors are involved in the development of equine pastern dermatitis (EPD). Among the most frequently suspected is *Staphylococcus aureus*, known for its pathogenic potential in skin and soft tissue infections. We therefore investigated the association between *S. aureus* carriage and EPD. One hundred five EPD-affected horses and 95 unaffected controls were examined for the presence of methicillin-resistant and -susceptible *Staphylococcus aureus* (MRSA and MSSA) on the pastern skin and in the nostrils. *S. aureus* isolates were cultivated from swab samples on selective MSSA and MRSA chromogenic agar and identified using MALDI-TOF MS. Isolates were analysed by Illumina whole genome sequencing for genetic relatedness (cgMLST, *spa* typing), and for the presence of antimicrobial resistance and virulence determinants. A markedly higher proportion of samples from EPD-

affected horses proved positive for *S. aureus*, both from the pastern (59.0 % vs. 6.3 % in unaffected horses; *P*<0.001), and from the nose (59.0 % vs. 8.4 %; *P*<0.001). Isolates belonged to 20 sequence types (ST) with lineages ST15-t084 (*spa*) (18 %), ST1-t127 (13 %), and ST1-t1508 (12 %) being predominant. Eight *S. aureus* were MRSA ST398-t011 and ST6239-t1456, and contained the staphylococcal cassette chromosome SCC*mec*IVa. Antimicrobial resistance genes were almost equally frequent in pastern and in nasal samples, whereas some virulence factors such as the beta-hemolysin, ESAT-6 secretion system, and some enterotoxins were more abundant in isolates from pastern samples, possibly enhancing their pathogenic potential. The markedly higher prevalence of *S. aureus* containing specific virulence factors in affected skin suggests their contribution in the development and course of EPD.

2.2.13 Comparative genomics of 26 complete circular genomes of 18 different serotypes of *Actinobacillus pleuropneumoniae*

Publication: Valentina Donà, Alban Ramette,Vincent Perreten (2022) Microb Genom. 8(2):000776, DOI: 10.1099/mgen.0.00077

Collaborator: Institute for Infectious Diseases, University of Bern, Bern, Switzerland

Abstract: Actinobacillus pleuropneumoniae is a Gram-negative, rod-shaped bacterium of the family *Pasteurellaceae* causing pig pleuropneumonia associated with great economic losses worldwide. Nineteen serotypes with distinctive lipopolysaccharide (LPS) and capsular (CPS) compositions have been described so far, yet complete circular genomes are publicly available only for the reference strains of serotypes 1, 4 and 5b, and for field strains of serotypes 1, 3, 7 and 8. We aimed to complete this picture by sequencing the reference strains of 17 different serotypes with the MinION sequencer (Oxford Nanopore Technologies, ONT) and on an Illumina HiSeq (Illumina) platform. We also included two field isolates of serotypes 2 and 3 that were PacBio- and MinION-sequenced, respectively. Genome assemblies were performed following two different strategies, i.e. PacBio- or ONT-only *de novo* assemblies polished with Illumina reads or a hybrid assembly by directly combining ONT and Illumina reads. Both methods proved successful in obtaining accurate circular genomes with comparable qualities. blast-based genome comparisons and core-genome phylogeny based on core genes, SNP typing and multi-locus sequence typing (cgMLST) of the 26 circular genomes indicated well-conserved genomes across the 18 different serotypes, differing mainly in phage insertions, and CPS, LPS and RTX-toxin clusters, which, consistently, encode serotype-specific antigens. We also identified small antibiotic resistance plasmids, and complete subtype I-F and subtype II-C CRISPR-Cas systems. Of note, highly similar clusters encoding all those serotype-specific traits were also found in other pathogenic and commensal *Actinobacillus* species. Taken together with the presence of transposable elements surrounding these loci, we speculate a dynamic intra- and interspecies exchange of such virulence-related factors by horizontal gene transfer. In conclusion, our comprehensive genomics analysis provides useful information for diagnostic test and vaccine development, but also for whole-genome-based epidemiological studies, as

well as for the surveillance of the evolution of antibiotic resistance and virulence genes in *A. pleuropneumoniae*.

2.2.14 Whole-genome analyses reveal a novel prophage and cgSNPs-derived sublineages of *Brachyspira hyodysenteriae* ST196

Publication: Ana Belén García-Martín, Thomas Roder, Sarah Schmitt, Friederike Zeeh, Rémy Bruggmann, Vincent Perreten (2022) BMC Genomics. 23(1):131. DOI: 10.1186/s12864-022-08347-5

Collaborators: Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland; Section of Veterinary Bacteriology, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; Clinic for Swine, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Abstract: Brachyspira (*B.*) *hyodysenteriae* is a fastidious anaerobe spirochete that can cause swine dysentery, a severe mucohaemorragic colitis that affects pig production and animal welfare worldwide. In Switzerland, the population of *B. hyodysenteriae* is characterized by the predominance of macrolide-lincosamide-resistant *B. hyodysenteriae* isolates of sequence type (ST) ST196, prompting us to obtain deeper insights into the genomic structure and variability of ST196 using pangenome and whole genome variant analyses. The draft genome of 14 *B. hyodysenteriae* isolates of ST196, sampled during a 7-year period from geographically distant pig herds, was obtained by whole-genome sequencing (WGS) and compared to the complete genome of the *B. hyodysenteriae* isolate Bh743-7 of ST196 used as reference. Variability results revealed the existence of 30 to 52 single nucleotide polymorphisms (SNPs), resulting in eight sublineages of ST196. The pangenome analysis led to the identification of a novel prophage, *pphBh*CH20, of the *Siphoviridae* family in a single isolate of ST196, which suggests that horizontal gene transfer events may drive changes in genomic structure. This study contributes to the catalogue of publicly available genomes and provides relevant bioinformatic tools and information for further comparative genomic analyses for *B. hyodysenteriae*. It reveals that Swiss *B. hyodysenteriae* isolates of the same ST may have evolved independently over time by point mutations and acquisition of larger genetic elements. In line with this, the third type of mobile genetic element described so far in *B. hyodysenteriae*, the novel prophage *pphBh*CH20, has been identified in a single isolate of *B. hyodysenteriae* of ST196.

2.2.15 *Macrococcus armenti* sp. nov., a novel bacterium isolated from the skin and nasal cavities of healthy pigs and calves

Publication: Jennifer E. Keller, Sybille Schwendener, Gudrun Overesch, Vincent Perreten (2022) Int J Syst Evol Microbiol. 72(2). DOI: 10.1099/ijsem.0.005245

Abstract: Gram-positive coccoid bacteria were isolated from the nasal cavities of pigs and calves as well as from axillar and inguinal skin regions of pigs. Phylogenetic analysis of seven strains based on complete genome, 16S rRNA, *hsp60*, *dnaJ*, *rpoB* and *sodA* gene sequences and

MALDI-TOF MS profiles revealed that they belonged to the genus *Macrococcus* with the closest relatedness to *Macrococcus canis*, *Macrococcus caseolyticus* subsp. *caseolyticus* and *Macrococcus caseolyticus* subsp. *hominis*. DNA relatedness of the type strain JEK37^T with the type strains of *M. canis*, *M. caseolyticus* subsp. *caseolyticus* and *M. caseolyticus* subsp. *hominis* was 23.4, 23.1 and 23.0 % by digital DNA-DNA hybridization and 80.39, 80.45 and 80.87 % by average nucleotide identity (ANI) calculations, confirming that they do not belong to the same species. The DNA G+C content of JEK37^T was 35.65 mol%. The novel strains can be differentiated from *M. canis* KM 45013^T by the ability to fermentate d-ribose and by the absence of DNAase production and haemolysis, from *M. caseolyticus* subsp. *caseolyticus* CCUG 15606^T by the ability to fermentate sucrose and from both species by the inability to grow in 9 and 12% NaCl. They differ from *M. caseolyticus* subsp. *hominis* by the presence of α-glucosidase. The most common fatty acids of JEK37^T were $C_{14:0}$, $C_{18:1}$ ω9*c* and $C_{18:0}$. Known polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, aminolipid, aminoglycolipid, aminophospholipid, glycolipid and phospholipid. Cell-wall peptidoglycan of JEK37^T was of type A3α l-Lys-Gly_2-L-Ser-Gly (similar to A11.3) and the respiratory quinolone was menaquinone 6. Based on their genotypic and chemotaxonomic characteristics, these strains represent a novel species of the genus *Macrococcus*, for which we propose the name *Macrococcus armenti* sp. nov. The type strain is JEK37^T (=DSM 112712^T=CCOS 1982^T).

2.2.16 Prevalence and characterization of methicillin-resistant *Macrococcus* spp. in food producing animals and meat in Switzerland in 2019

Publication: Jennifer E. Keller, Sybille Schwendener, Jil Neuenschwander, Gudrun Overesch, Vincent Perreten (2022) Schweiz Arch Tierheilkd. 164(2):153-164. DOI: 10.17236/sat00343

Abstract: The prevalence of methicillin-resistant *Macrococcus* spp. in calves and pigs at slaughterhouses and in retail beef and pork meat was determined using samples taken in 2019 within the framework of the national monitoring of methicillin-resistant *Staphylococcus aureus* in food producing animals in Switzerland. The isolates were submitted to antimicrobial susceptibility testing of 19 antibiotics and to molecular techniques (e.g. PCR, microarray, WGS) for the identification of resistance genes, elements containing the methicillin resistance genes mec and sequence type (ST). Methicillin-resistant *Macrococcus* spp. (*M. caseolyticus* (n=38), *M. bohemicus* (n=4) and *Macrococcus* spp. (n=2)) were isolated in 40 of 299 nasal swabs from calves representing a prevalence of 13,38 % (95 % CI, 9,98 % - 17,70 %), and in four of 303 nasal swabs from pigs [1,32 % (95 % CI, 0,36 % - 3,35 %)]. One of 311 samples of Swiss pork meat contained a *Macrococcus* sp. [0,32 % (95 % CI, 0,01 % - 1,78 %)], and four of 309 beef meat samples (260 domestic and 49 imported) contained *M. caseolyticus* [1,29 % (95 % CI, 0,35 % - 3,28 %)]. The *M. caseolyticus* strains belonged to diverse STs, with ST21 being the most common in both pigs and calves. The *mecD* gene was located on *Macrococcus* resistance island mecD (McRImecD) in 42 strains and on staphylococcal cassette chromosome mec (SCC_{mecD}) in three strains, while *mecB* was found on plasmids in four strains. In addition

to resistance to β-lactams, the strains also exhibited resistance to tetracycline (n=17; *tet*(L), *tet*(K), *tet*(M)), streptomycin (n=13; *str*, *ant(6)-Ia*, *rpsL* mutation [K56R in ribosomal protein S12]), kanamycin (n=10; *aac(6')-Ie - aph(2'')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *ant(4')-Ia*), clindamycin (n=9; *erm*(B), *erm*(45)), erythromycin (n=9; *erm*(B), *msr*(G), *erm*(45)), fusidic acid (n=9; *fusC*) and gentamicin (n=1; *aac(6')-Ie - aph(2'')-Ia*). This study represents the first national prevalence study of methicillin-resistant *Macrococcus* spp. in pigs, calves, pork and beef meat in Switzerland and revealed a reservoir of genetically diverse strains carrying several resistance traits.

2.2.17 Addition of daptomycin to levofloxacin increased the efficacy of levofloxacin monotherapy against a methicillin-susceptible *Staphylococcus aureus* strain in experimental meningitis and prevented development of resistance in vitro

Publication: Philippe Cottagnoud, Frederike Sprenker, Marianne Cottagnoud, Alexandra Collaud, Reza Ashkbus, Vincent Perreten (2022) J Med Microbiol. 71(2). DOI: 10.1099/jmm.0.001497

Collaborators: Ärztezentrum Südbahnhof, Bern, Switzerland; Former Spitalnetz Bern, Bern, Switzerland

Abstract: Daptomycin and levofloxacin were tested as monotherapies and in combination against the antibiotic-susceptible *S. aureus* strain MSSA 1112 in a rabbit meningitis model and the effect of the combination on induction of resistance was determined in vitro. The aim of the study was to demonstrate a synergy of the antibiotic combination daptomycin and levofloxacin in experimental meningitis, and also its ability to reduce the emergence of resistance against the antibiotics in vitro. Changes of the susceptibility to fluoroquinolones and daptomycin were determined by the measurement of the MIC and mutations were detected by whole genome sequence comparison of the mutants with the parent strain MSSA 1112. Meningitis was induced by intracisternal inoculation of 105 c.f.u. (colony forming unit) of MSSA 1112 and treatment was started 10 h later by injection of daptomycin (15 mg kg^{-1}) and levofloxacin (10 mg kg^{-1}) standard doses. Cerebrospinal fluid (CSF) samples were repeatedly collected during therapy in order to determine killing rates and results of bactericidal activity were expressed in Δlog10c.f.u. ml^{-1} over 8 h. The combination of daptomycin with levofloxacin was significantly ($P<0.001$) superior to levofloxacin monotherapy and increased the antibacterial activity of daptomycin. In vitro, MSSA 1112 was cycled over 6 days with either increasing concentrations of levofloxacin or daptomycin or with a combination of levofloxacin with half of the MIC of daptomycin or daptomycin with half of the MIC of levofloxacin leading to mutations in target genes as identified by whole genome sequence analysis. Addition of low concentration of daptomycin (0.25 mg l^{-1}) reduced levofloxacin-induced resistance in vitro. Addition of levofloxacin in low concentration (0.125 mg l^{-1}) did not influence daptomycin-induced resistance. These findings highlight the lack of reciprocal interference of antibiotics in combination with regard to the development of resistance.

2.2.18 Targeted Genome Mining Reveals the Psychrophilic *Clostridium estertheticum* Complex as a Potential Source for Novel Bacteriocins, Including Cesin A and Estercticin A

Publication: Joseph Wambui, Marc J A Stevens, Simon Sieber, Nicole Cernela, Vincent Perreten, Roger Stephan (2022) Front Microbiol. 12:801467, DOI: 10.3389/fmicb.2021.801467
Collaborators: Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; Department of Chemistry, University of Zurich, Zurich, Switzerland
Abstract: Antimicrobial resistance in pathogenic bacteria is considered a major public health issue necessitating the discovery of alternative antimicrobial compounds. In this regard, targeted genome mining in bacteria occupying under-explored ecological niches has the potential to reveal such compounds, including bacteriocins. In this study, we determined the bacteriocin biosynthetic potential of the psychrophilic *Clostridium estertheticum* complex (CEC) through a combination of genome mining and phenotypic screening assays. The genome mining was performed in 40 CEC genomes using antiSMASH. The production of bacteriocin-like compounds was phenotypically validated through agar well (primary screening) and disk diffusion (secondary screening) assays using cell free supernatants (CFS) and partially purified extracts, respectively. Stability of four selected CFS against proteolytic enzymes, temperature and pH was determined while one CFS was analyzed by HRMS and MS/MS to identify potential bacteriocins. Twenty novel bacteriocin biosynthetic gene clusters (BBGC), which were classified into eight (six lantibiotics and two sactipeptides) distinct groups, were discovered in 18 genomes belonging to *C. estertheticum* (*n* = 12), *C. tagluense* (*n* = 3) and genomospecies2 (*n* = 3). Primary screening linked six BBGC with narrow antimicrobial activity against closely related clostridia species. All four preselected CFS retained activity after exposure to different proteolytic, temperature and pH conditions. Secondary screening linked BBGC1 and BBGC7 encoding a lantibiotic and sactipeptide, respectively, with activity against *Bacillus cereus* while lantibiotic-encoding BBGC2 and BBGC3 were linked with activity against *B. cereus*, *Staphylococcus aureus* (methicillin-resistant), *Escherichia coli* and *Pseudomonas aeruginosa*. MS/MS analysis revealed that *C. estertheticum* CF004 produces cesin A, a short natural variant of nisin, and HRMS indicated the production of a novel sactipeptide named estercticin A. Therefore, we have shown the CEC, in particular *C. estertheticum*, is a source of novel and stable bacteriocins that have activities against clinically relevant pathogens.

2.2.19 Carbapenemase-producing *Klebsiella pneumoniae* strains in Switzerland: human and non-human settings may share high-risk clones

Publication: Edgar I Campos-Madueno, Aline I Moser, Géraldine Jost, Carola Maffiol, Thomas Bodmer, Vincent Perreten, Andrea Endimiani (2022) J Glob Antimicrob Resist. 28:206-215, DOI: 10.1016/j.jgar.2022.01.016.

Collaborators: Institute for Infectious Diseases, University of Bern, Bern, Switzerland; Dianalabs, Geneva, Switzerland; MCL Medizinische Laboratorien, Niederwangen, Switzerland; Medical Microbiology, Dr Risch Medical Laboratories, Bern-Liebefeld, Switzerland

Abstract: The spread of carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) strains belonging to high-risk sequence types (STs) is a concern. For Switzerland, national data about the molecular features (especially the STs) of CP-Kp of human origin is not available. In veterinary clinics, ST11 and ST307 *bla*$_{\text{OXA-48}}$-possessing *K. pneumoniae* strains have been recently reported. A collection of 285 *K. pneumoniae* genomes (170 were CP-Kp) isolated in Switzerland from human and non-human sources during 2006-2020 have been analyzed. Whole-genome sequencing, core genome phylogenies and public databases were used to present a detailed overview regarding carbapenemases, STs and plasmids. The top five STs were (main carbapenemase gene) ST512 (*bla*$_{\text{KPC-3}}$), ST258 (*bla*$_{\text{KPC-2}}$) and ST101 (*bla*$_{\text{OXA-48}}$), consisting of strains of human origin only, and ST11 (*bla*$_{\text{OXA-48}}$) and ST307 (*bla*$_{\text{OXA-48}}$) strains isolated from human, animal and environmental sources. However, during 2016-2020, the main STs for CP-Kp were ST11 (17.6%), ST307 and ST101 (both 14.7%), whereas ST258 (5.9%) and ST512 (4.4%) significantly declined. Most carbapenemase genes were carried on plasmids already described. Core genome analysis revealed that ST11 *K. pneumoniae* of animal and human origin were closely related, whereas those of ST307 were distant. Our genomic analysis revealed that the emerging high-risk ST11 and ST307 lineages were often isolated from non-human settings. This study provided a baseline for further whole-genome sequencing-based One-Health surveillance of CP-Kp and emphasized the need for metadata to track dissemination routes between the different settings.

2.2.20 The dose makes the poison: feeding of antibiotic-treated winter honey bees, Apis mellifera, with probiotics and b-vitamins

Publication: Andrew Brown, Victor Rodriguez, Judith Pfister, Vincent Perreten, Peter Neumann, Gina Retschnig (2022) Apidologie 53:19, DOI: 10.1007/s13592-022-00927-4

Collaborators: Institute of Bee Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Agroscope, Swiss Bee Research Centre, Bern, Switzerland

Abstract: Honey stores of *Apis mellifera* colonies are replaced with sugar water by beekeepers, which may result in malnutrition. Nutritional supplements have been developed, but the importance of bacterial probiotics and vitamins is poorly understood. Given that supplementary feeding with vitamins and probiotics may enhance worker weight and longevity, this might suggest a feasible approach to mitigate winter colony losses. Here, we conducted a laboratory hoarding cage study with freshly emerged winter bees, which were treated with the antibiotic tetracycline to reduce gut bacteria obtained post-emergence and subsequently assigned to feeding regimes: sucrose only, sucrose + pollen, probiotics (low and high dosage), probiotics + pollen (low and high dosage), or b-vitamins (low and high dosage) ($N = 8$

treatments, 29 workers/cage × 8 replicates). In parallel, another age cohort of bees remained on their frame (= Frame) to establish their gut microbiota and were subsequently fed with sucrose only or sucrose + pollen ($N = 2$ treatments, 29 workers/cage × 4 replicates). The most beneficial effects on body weights were found in workers given *ad libitum* access to pollen, notably in the Frame Sucrose + Pollen group, confirming the inherent importance of post-emergent gut flora inoculation and the role of gut bacteria in protein digestion. Furthermore, both Frame groups and the antibiotic-treated workers fed with probiotic low + pollen survived longer than all other groups, highlighting a fundamental host-microbial relationship. On the other hand, our current treatments alone, post-tetracycline, did not yield any positive results. In contrast, high dosages of both probiotic and b-vitamins significantly reduced lifespan compared to their low concentration counterparts, probably due to dysbiosis and toxicity, suggesting that the outcome was dose-dependent. These results highlight that bacterial and b-vitamin supplementation can alter longevity with advisable caution since harmful concentrations appear to exist.

2.2.21 Emergence of methicillin resistance predates the clinical use of antibiotics

Publication: Jesper Larsen, Claire L Raisen, Xiaoliang Ba, Nicholas J Sadgrove, Guillermo F Padilla-González, Monique S J Simmonds, Igor Loncaric, Heidrun Kerschner, Petra Apfalte, Rainer Hartl, Ariane Deplano, Stien Vandendriessche, Barbora Černá Bolfíková, Pavel Hulva, Maiken C Arendrup, Rasmus K Hare, Céline Barnadas, Marc Stegger, Raphael N Sieber, Robert L Skov, Andreas Petersen, Øystein Angen, Sophie L Rasmussen, Carmen Espinosa-Gongora, Frank M Aarestrup, Laura J Lindholm, Suvi M Nykäsenoja, Frederic Laurent, Karsten Becker, Birgit Walther, Corinna Kehrenberg, Christiane Cuny, Franziska Layer, Guido Werner, Wolfgang Witte, Ivonne Stamm, Paolo Moroni, Hannah J Jørgensen, Hermínia de Lencastre, Emilia Cercenado, Fernando García-Garrote, Stefan Börjesson, Sara Hæggman, Vincent Perreten, Christopher J Teale, Andrew S Waller, Bruno Pichon, Martin D Curran, Matthew J Ellington, John J Welch, Sharon J Peacock, David J Seilly, Fiona J E Morgan, Julian Parkhill, Nazreen F Hadjirin, Jodi A Lindsay, Matthew T G Holden, Giles F Edwards, Geoffrey Foster, Gavin K Paterson, Xavier Didelot, Mark A Holmes, Ewan M Harrison, Anders R Larsen (2022) Nature. 602(7895):135-141. DOI: 10.1038/s41586-021-04265-w

Collaborators: Department of Bacteria, Parasites & Fungi, Statens Serum Institut, Copenhagen, Denmark; Department of Veterinary Medicine, University of Cambridge, Cambridge, UK; Royal Botanic Gardens, Kew, Richmond, UK; Institute of Microbiology, University of Veterinary Medicine, Vienna, Austria; National Reference Center for Antimicrobial Resistance and Nosocomial Infections, Institute for Hygiene, Microbiology and Tropical Medicine, Ordensklinikum Linz Elisabethinen, Linz, Austria; National Reference Centre-Staphylococcus aureus, Department of Microbiology, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium; Laboratory for Medical Microbiology, Ghent University Hospital, Ghent, Belgium; Department of Animal Science and Food Processing, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague, Czech Republic; Department of Zoology, Charles

University, Prague, Czech Republic; Department of Biology and Ecology, University of Ostrava, Ostrava, Czech Republic; Department of Bacteria, Parasites & Fungi, Statens Serum Institut, Copenhagen, Denmark; European Programme for Public Health Microbiology Training (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden; Infectious Disease Preparedness, Statens Serum Institut, Copenhagen, Denmark; Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark; Wildlife Conservation Research Unit (WildCRU), Department of Zoology, University of Oxford, Tubney, UK; Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark; National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark; Expert Microbiology Unit, Department of Health Security, Finnish Institute for Health and Welfare, Helsinki, Finland; Microbiology Unit, Finnish Food Authority, Helsinki, Finland; Bacteriology Department and French National Reference Center for Staphylococci, Hospices Civils de Lyon, University of Lyon, Lyon, France; Friedrich Loeffler-Institute of Medical Microbiology, University Medicine Greifswald, Greifswald, Germany; Institute of Microbiology and Epizootics, Veterinary Faculty, Freie Universität Berlin, Berlin, Germany; Advanced Light and Electron Microscopy (ZBS-4), Robert Koch Institute, Berlin, Germany; Institute for Veterinary Food Science, Justus-Liebig University Giessen, Giessen, Germany; National Reference Centre for Staphylococci and Enterococci, Division Nosocomial Pathogens and Antibiotic Resistances, Department of Infectious Diseases, Robert Koch Institute, Wernigerode, Germany; Vet Med Labor GmbH, Kornwestheim, Germany; Dipartimento di Medicina Veterinaria, Università degli Studi di Milano, Lodi, Italy; Quality Milk Production Services, Animal Health Diagnostic Center, Cornell University, Ithaca, NY, USA; Norwegian Veterinary Institute, Ås, Norway; Laboratory of Molecular Genetics, ITQB NOVA, Oeiras, Portugal; Laboratory of Microbiology and Infectious Diseases, The Rockefeller University, New York, NY, USA; Servicio de Microbiología, Hospital Universitario Lucus Augusti, Lugo, Spain; Servicio de Microbiología, Complejo Asistencial Universitario de Salamanca, Salamanca, Spain; Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute (SVA), Uppsala, Sweden; Department of Microbiology, Public Health Agency of Sweden, Solna, Sweden; Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland; Department of Bacteriology, Animal and Plant Health Agency, Weybridge, UK; Animal Health Trust, Newmarket, UK; Intervacc AB, Stockholm, Stockholm, Sweden; Department of Biomedical Science and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden; Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, UK Health Security Agency, London, UK; Clinical Microbiology and Public Health Laboratory, UK Health Security Agency, Addenbrooke's Hospital, Cambridge, UK

Abstract: The discovery of antibiotics more than 80 years ago has led to considerable improvements in human and animal health. Although antibiotic resistance in environmental bacteria is ancient, resistance in human pathogens is thought to be a modern phenomenon that

is driven by the clinical use of antibiotics. Here we show that particular lineages of methicillin-resistant *Staphylococcus aureus*-a notorious human pathogen-appeared in European hedgehogs in the pre-antibiotic era. Subsequently, these lineages spread within the local hedgehog populations and between hedgehogs and secondary hosts, including livestock and humans. We also demonstrate that the hedgehog dermatophyte Trichophyton erinacei produces two β-lactam antibiotics that provide a natural selective environment in which methicillin-resistant S. aureus isolates have an advantage over susceptible isolates. Together, these results suggest that methicillin resistance emerged in the pre-antibiotic era as a co-evolutionary adaptation of S. aureus to the colonization of dermatophyte-infected hedgehogs. The evolution of clinically relevant antibiotic-resistance genes in wild animals and the connectivity of natural, agricultural and human ecosystems demonstrate that the use of a One Health approach is critical for our understanding and management of antibiotic resistance, which is one of the biggest threats to global health, food security and development.

3 ZOBA – Centre for Zoonoses, Bacterial Epizootics and Antimicrobial Resistance

In ZOBA encompasses the two subdivisions (i) 'Diagnostic Services and Epizootic Surveillance'and (ii) 'Reference Laboratories and Antimicrobial Resistance Monitoring'. The two subdivisons performed a total of 22054 analyses. Details are shown in Table 1.

Table 1: Number of investigated samples listed per unit of the two subdivisions

Subdivision	Unit	Number of analyses
Diagnostic Services and Epizootic Surveillance	Clinical material and mycology	3273
	Necropsy material, abortion and feaces	2859
	Molecular diagnostics (PCR incl. qPCR)	1830
	Bovine mastitis	4095
	Serology	2411
	Antibiograms for diagnostics	2120
Reference Laboratories	Reference laboratories	268
Antimicrobial Resistance Monitoring	European monitoring in livestock and fresh meat thereof (Detection)	3812
	European monitoring in livestock and fresh meat thereof (number of isolates for MIC*)	685
	Swiss monitoring of animal pathogens (number of isolates for MIC)	701

* Minimal inhibitory concentration

3.1 Diagnostic Services and Epizootic Surveillance (Notifiable Animal Diseases)

Methods:

Micr	Microscopic examination
IF	Immunofluorescence
IA	Immunoassay
Cult	Culture
ELISA	Antibody detection by Enzyme-Linked Immunosorbent Assay
RBT	Antibody detection by Rose Bengal test

CFT Antibody detection by complement fixation test

MAT Antibody detection by the microscopic agglutination test

PCR Polymerase chain reaction

SEQ Sequencing

ST Serotyping (* in human reference laboratory)

3.1.1 Highly infectious epizootics

Table 2: Number of samples investigated related to highly infectious epizootics

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Contagious bovine pleuropneumonia	*Mycoplasma mycoides* subsp. *mycoides*	Cult	**Cattle**	0	0	0	0
		PCR		0	0	0	0
		ELISA		0	0	0	0
Glanders	*Burkholderia mallei*	CFT	**Horse**	0	0	0	0
		Cult		0	0	0	0

3.1.2 Epizootics to be eradicated

Table 3: Number of samples investigated related to epizootics to be eradicated

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Anthrax	*Bacillus anthracis*	Micr	**Cattle**	4	4	0	0
		Cult		4	4	0	0
Brucellosis	*Brucella abortus/melitensis/suis*	Micr	**Cattle**	54	53	1	0
		RBT		0	0	0	0
		ELISA		814	814	0	0
		CFT		0	0	0	0
		Cult		2	2	0	0
		PCR		3	3	0	0
	Brucella abortus/melitensis/suis	Micr	**Sheep/goat**	40	35	5	0
		PCR		0	0	0	0
		ELISA		20	20	0	0
		CFT		0	0	0	0
		RBT		1	1	0	0

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Brucellosis	*Brucella*	Micr	**Others**	30	28	2	0
	abortus/melitensis/suis	PCR		4	4	0	0
		ELISA		21	21	0	0
		CFT		0	0	0	0
		RBT		54	54	0	0
	Brucella	Micr	**Pig**	18	18	0	0
	abortus/melitensis/suis	PCR	Incl. wild boar	8	7	0	1*
	*wild boar	Cult	Incl. wild boar	7	6	0	1*
		RBT		895	887	1	7
		ELISA		8	7	0	1
		R/S ELISA		7	7	0	0
		CFT		7	6	1	0
	Brucella ovis (epizootic to be controlled)	ELISA	**Sheep**	16	13	0	3
	Brucella canis	Micr	**Dog**	8	8	0	0
	(no epizootic)	IA		14	13	0	1
		PCR		13	13	0	0
		Cult		4	4	0	0
Bovine genital Campylo-bacteriosis	*Campylobacter fetus*	Cult	**Cattle**	774	774	0	0
	subspecies *venerealis*	PCR		217	217	0	0
Sporadic *Campylobacter* abortion	*Campylobacter fetus* subspecies *fetus*	Cult	**Ruminants**	64	64	0	0
	(no epizootic)	PCR		0	0	0	0

3.1.3 Epizootics to be controlled

Table 4: Number of samples investigated related to epizootics to be controlled

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Leptospirosis	*L.* Australis	MAT	**Dog**	70	54	12	4
			Cattle	674	672	2	0
			Others	16	12	0	4
	L. Autumnalis	MAT	**Dog**	70	64	4	2
			Others	9	5	0	4
	L. Ballum	MAT	**Dog**	7	7	0	0
			Cattle	45	44	1	0
			Others	7	7	0	0
	L. Bataviae	MAT	**Dog**	70	69	1	0
			Others	9	5	0	4
	L. Bratislava	MAT	**Cattle**	17	17	0	0
			Dog	70	64	2	4
			Others	7	7	0	0
	L. Canicola	MAT	**Cattle**	674	674	0	0
			Dog	70	69	1	0
			Others	16	16	0	0
	L. Copenhageni	MAT	**Dog**	70	58	8	4
			Others	2	2	0	0
	L. Grippotyphosa	MAT	**Cattle**	676	676	0	0
			Dog	70	62	7	1
			Others	16	15	0	1
	L. Hardjo	MAT	**Cattle**	681	640	25	16
			Dog	70	69	1	0
			Others	4	4	0	0
	L. Icterohaemo-rrhagiae	MAT	**Cattle**	674	674	0	0
			Dog	70	69	1	0
			Others	16	12	0	4
	L. Pomona	MAT	**Cattle**	674	673	1	0
			Dog	70	62	6	2

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Leptospirosis	*L.* Pomona	MAT	**Others**	16	12	0	4
	L. Pyrogenes	MAT	**Dog**	70	70	0	0
			Others	9	4	5	0
	L. Sejroe	MAT	**Cattle**	678	659	19	0
			Dog	7	7	0	0
			Others	2	2	0	0
	L. Tarassovi	MAT	**Cattle**	45	44	1	0
			Dog	70	70	0	0
	L. Tarassovi	MAT	**Others**	16	16	0	0
	Leptopsira spp. (pathogen)	MAT Eye	**Donkey**	12	12	0	0
	Leptopsira spp. (pathogen)	PCR	**Beaver**	14	3	0	11
			Cattle	8	5	0	3
			Dog	12	12	0	0
			Horse	1	1	0	0
			Others	24	21	0	3
Salmonellosis	*Salmonella* spp.	Cult/ST	**Alpaca**	10	10	0	0
			Ape	14	14	0	0
			Beaver	1	1	0	0
			Bison	2	2	0	0
			Bird	81	80	0	1
	S. Hessarek						1
			Cat	30	29	0	1
	S. Mishmarheamek						1
			Cattle	405	371	0	34
	S. Dublin						13
	S. Enteritidis						21
			Chamois	1	1	0	0
			Chicken	19	19	0	0
			Deer	2	2	0	0
			Dog	40	38	0	2
	S. Enteritidis						1

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Salmonellosis	*S.* Veneziana	Cult/ST	**Dog**				1
			Fox	3	2	0	1
	S. Enteritidis						1
			Goat	21	21	0	0
			Hedgehog	1	0	0	1
	S. Enteritidis						1
			Horse	111	102	0	9
	S. Enteritidis						3
	S. Typhimurium						3
	S. Veneziana						3
			Rabbit	9	8	0	1
	S. Typhimurium						1
			Reptil	30	25	0	5
	S. Abaetetuba						1
	S. Fresno						1
	S. enterica subsp. *enterica* rough:i:1,5						1
	S. enterica subsp. *diarizonae* 48:r:z*						1
	S. enterica subsp. *salamae* 1,9,12,46,27:l,z13,z28:z39*						1
			Sheep	24	21	0	3
	S. enterica subsp. *diarizonae* 61: k: 1,5,7						3
			Snake	21	11	0	10
	S. Aqua						1
	S. enterica subsp. *arizonae* rough:g:-*						1
	S. enterica subsp. *diarizonae* 61:i:z35*						1
	S. enterica subsp. *diarizonae* 48:c:z*						1
	S. enterica subsp. *diarizonae* rough: r:z						1

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Salmonellosis	*S. enterica* subsp. *diarizonae* 6,14:z10:z	Cult/ST	**Snake**				1
	S. enterica subsp. *diarizonae* 11:l,v:z*						2
	S. Parathyphi B (d-Tartrat positiv; ehemals Java)						1
	S. enterica subsp. *houtenae* 53:g,z51:-						1
			Pig	44	44	0	0
			Tortoise	10	10	0	0
			Wild Boar	1	1	0	1
	S. enterica subsp. e*nterica* rough:g,m:-						1
			Wolf	1	1	0	0
			Zoo animal	59	59	0	0
			Total	**943**	**872**	**0**	**144**
Contagious equine metritis	*Taylorella equigenitalis*	Cult	**Horse**	554	506	46*	2
			Donkey	12	9	3*	0
		PCR	**Horse**	59	59	0	0
* highly contaminated.			**Donkey**	3	3	0	0
Enzootic pneumonia in swine	*Mycoplasma hyopneumoniae*	PCR Lung (pooled)	**Pig**	56	54	0	2
		PCR Lung (single)		7	7	0	0
		PCR Nasal swabs (pooled)		25	23	0	2
		PCR Nasal swabs (single)		2	1	0	1
		PCR Lung (single)	**Wild boar**	3	0	0	3
		MLST	**Pig**	2	0	0	2
		MLST	**Wild boar**	5	0	0	5
		ELISA	**Pig**	42	33	3	6

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Swine actinobacillosis	*Actinobacillus pleuropneumoniae*	Cult	**Pig**	166	100	13*	53
* highly contaminated	I BD +II CA, Serotyp 7,12	PCR					30
	I BD+II CA+III CA+BD, Serotyp 2	PCR	**Pig**				18
	II CA+IIICA+BD, Serotyp 3						5
		ELISA ApxIV	**Pig**	4	1	0	3

3.1.4 Epizootics to be Monitored

Table 5: Number of samples investigated related to epizootics to be monitored

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Campylobac-teriosis	*Campyobacter* spp.	Cult	**Dog**	27	25	0	2
			Cat	27	23	0	4
			Others	48	34	0	14
Listeriosis	*Listeria*	Cult	**Ruminants**	3	3	0	0
	monocytogenes	PCR		2	2	0	0
		Cult	**Others**	7	4	0	3
		PCR		0	0	0	0
Yersiniosis	*Y. enterocolitica*	Cult	**Diverse**	33	32	0	1
	Y. pseudotuberculosis		**Diverse**	21	19	0	3
	Y. ruckeri		**Fish**	0	0	0	0
Caseous lymphadenitis in sheep/goat	*Corynebacterium pseudotuberculosis*	Cult	**Goat**	1	0	0	1
			Sheep	0	0	0	0
Enzootic abortion in ewes	*Chlamydia abortus*	PCR	**Sheep**	37	26	2	9
		PCR	**Cattle**	18	18	0	0
		PCR	**Goat**	20	19	0	1
		PCR	**Others**	10	2	6	0
Psittacosis	*Chlamydia psittaci*	PCR	**Bird**	3	3	0	0
Tularaemia	*Francisella*	Cult	**Hare**	35	19	0	16
	tularensis	PCR		15	11	0	4

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Tularaemia	*Francisella*	Cult	**Others**	11	11	0	0
	tularensis	PCR		10	10	0	0
Blackleg	*Clostridium*	IF	**Ruminant**	4	4	0	0
	chauvoei	Cult		4	3	0	1
Coxiellosis	*Coxiella burnetii*	ELISA	**Cattle**	30	28	0	2
Coxiellosis	*Coxiella burnetii*	PCR	**Cattle**	23	19	0	4
		ELISA	**Sheep**	51	51	0	0
		PCR	**Sheep**	37	33	0	4
		PCR	**Goat**	21	17	1	3

3.1.5 Epizootics planned to be eradicated in the future

Table 5: Number of samples investigated

Epizootics	Method	Host	Agent	Total	Negative	Suspicious	Positive
Foot rot	PCR (single or pool)	**Sheep**	*benigne D. nodosus*	49	48	0	1
			virulent D. nodosus	49	41	0	8
		Goat	*benigne D. nodosus*		3	0	0
			virulent D. nodosus	3	3	0	0

3.2 Reference Laboratories and Resistance Monitoring

3.2.1 Antimicrobial resistance monitoring for food producing animals and meat thereof

The program follows the new specifications laid down in the decision (EU) 2020/1729 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria in Europe from 2021 on. Caecal samples from chicken were collected at slaughter and cultured for indicator *Escherichia* (*E.*) *coli*, *Campylobacter* (*C.*) *jejuni* and *C. coli*, extended spectrum beta-lactamases producing *E. coli* (ESBLs) and carbapenemases producing *E. coli* and *Klebsiella* spp.. Moreover, fresh chicken meat and – for the first time – fresh turkey meat from retail was analysed for ESBLs and carbapenemases producing *E. coli* and *Klebsiella* spp.. Isolated strains and all *Salmonella enterica* subspecies *enterica* strains from poultry, provided either from diagnostics and reference function, were tested for antimicrobial susceptibility. Minimal inhibitory concentration (MIC) determination was performed by the broth microdilution method.

Results of this antimicrobial resistance monitoring get published in the biennial Swiss antibiotic resistance report, published by the Federal Office of Public Health (FOPH) and Federal Food Safety and Veterinary Office (FSVO). Moreover, a summary of the results are published in the annual ARCH-Vet reports, published by the Federal Food Safety and Veterinary Office (FSVO). On the European level the results are listed in the annual European summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food, published by the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC).

The numbers of analyses in the framework of the European harmonized antimicrobial resistance monitoring that were analyzed in 2022 are shown in tables 6a to 6e.

Table 6a: Number of analyses on ESBL/AmpC producing *E. coli*

Matrix	Number of analyses
Caecal samples from chicken	510
Chicken meat samples	307
Turkey meat samples	139
Total	**956**

Table 6b: Number of analyses on carbapenemases producing *E. coli* and *Klebsiella* spp.

Matrix	Number of analyses
Caecal samples from chicken	510
Chicken meat samples	307
Turkey meat samples	139
Total	**956**

Table 6c: Number of analyses on indicator *E. coli*

Matrix	Number of analyses
Caecal samples from chicken	240
Total	**240**

Table 6d: Number of analyses on *Campylobacter* spp.

Matrix	Number of analyses
Caecal samples from chicken	800 for *C. jejuni*
Caecal samples from chicken	800 for *C. coli*
Total	**1600**

Table 6e: Number of analyses on *Salmonella enterica* subspecies *enterica* from poultry

Salmonella serovar	Number of analyses
S. Enteritidis	15
S. Typhimurium	12
S. Typhimurium, monophasic variant	2
other serovars	31
Total	**60**

3.2.2 Antimicrobial resistance monitoring of animal pathogens

Monitoring of antimicrobial resistance for relevant pathogens from diseased livestock and companion animals is important for veterinarians, as it enables them to make appropriate therapeutic antibiotic choices, which they often cannot base on an antibiogram prior to the first treatment. Moreover, these data fill another important gap regarding monitoring of antimicrobial resistance from the One-Health perspective. In 2019, an annual monitoring of antimicrobial resistance in veterinary pathogens was initiated by the Federal Food Safety and Veterinary Office (FSVO) and implemented at the Swiss national reference laboratory for antimicrobial resistance (ZOBA). The sampling plan includes various pathogen/animal and indication combinations. For each isolate suceptibility against more than 20 antimicrobials are tested. Isolates isolated from clinical submissions of diseased animals by Swiss laboratories (university, cantonal, private) all over Switzerland are send to ZOBA. Susceptibility testing of all isolates is performed by broth microdilution, which guarantees fully comparability of data over time and with data from other European national resistance monitoring programs.

Minimal inhibitory concentrations are transmitted to the database of the Swiss centre for antimicrobial resistance (anresis), which is the nationwide system for resistance data for both, human and veterinary medicine (www.anresis.ch). By this, resistance rates are accessible on the interactive veterinary database query. Moreover, all raw and interpretative data are accessible via INFECT, which is an INterface For Empirical antimicrobial ChemoTherapy. This online tool provides a fast and intuitive access to the latest antimicrobial resistance data in Swiss veterinary pathogens for assisting veterinarians with reliable empirical treatment options (www.vet.infect.info). Results of this antimicrobial resistance monitoring get published in the biennial Swiss antibiotic resistance report, published by the Federal Office of Public Health (FOPH) and Federal Food Safety and Veterinary Office (FSVO). Moreover, a summary of the results are published in the annual ARCH-Vet reports, published by the Federal Food Safety and Veterinary Office (FSVO). The number of isolates analyzed in 2022 are listed in Table 7.

Table 7: Number of isolates analysed for the national resistance monitoring in animal pathogens

Animal species	Indication	Bacterial species	Number of isolates
Dairy	Mastitis	*Staphylococcus dysgalcatiae*	81
Dairy	Mastitis	*Trueperella pyogenes*	72
Dairy	Mastitis	Coagulase negative Staphylococci	101
Dog	Urogenital tract infection	*Escherichia coli*	120
Dog	Skin infection	*Staphylococcus pseudintermedius*	63
Cat	Urogenital tract infection	*Escherichia coli*	109
Cat	Skin infection	*Staphylococcus aureus*	9
Horse	Skin infection	*Staphylococcus aureus*	11
Small ruminant	Abscess	*Corynebacterium pseudotuberculosis*	16
Chicken	Divers	*Escherichia coli*	94
Cattle	Respiratory tract infection	*Mannheimia heamolytica*	11
Pig	Respiratory tract infection	*Pasteurella multocida*	13
Pig	Skin infection	*Staphylococcus hyicus*	1
Number of isolates which did not meet the criteria of the program			55
Total			**756**

3.2.3 Confirmation of results from other laboratories (exclusive typing for *Salmonella* spp. and *Actinobacillus pleuropneumoniae*)

Table 7: Number of analyses for confirmation of results from other laboratories (exclusive typing for *Salmonella* spp. and *Actinobacillus pleuropneumoniae*)

Epizootic	Method	Animal	Total	Negative	Suspicious	Positive
Anthrax	Micr		0	0	0	0
	Cult		0	0	0	0
Contagious bovine pleuropneumonia	Cult	**Cattle**	0	0	0	0
	PCR		0	0	0	0
Glanders	Cult	**Horse**	0	0	0	0
Bovine brucellosis	ELISA	**Cattle**	6	5	1	0
	RBT		6	6	0	0
	CFT		6	5	1	0
	Micr		0	0	0	0
	Cult		0	0	0	0
	PCR		0	0	0	0
Caprine and ovine brucellosis	ELISA	**Sheep/Goat**	0	0	0	0
	RBT		0	0	0	0
	CFT		0	0	0	0
Porcine brucellosis	ELISA multispecies	**Swine**	0	0	0	0
	ELISA B. suis		0	0	0	0
	RBT		0	0	0	0
	CFT		0	0	0	0
	Cult		0	0	0	0
	PCR		0	0	0	0
Canine brucellosis	LF	**Dog**	1	1	0	0
	Micr		0	0	0	0
	Cult		0	0	0	0
	Direct PCR		1	1	0	0
Ovine epididymitis (*Brucella ovis*)	ELISA	**Sheep**	0	0	0	0
Bovine genital campylobacteriosis	ID	**Cattle**	1	1	0	0

Epizootic	Method	Animal	Total	Negative	Suspicious	Positive
Contagious equine metritis	Cult	**Horse**	0	0	0	0
	ID		0	0	0	0
Blackleg	IF	**Cattle**	1	1	0	0
	Cult		3	3	0	0
	PCR		1	1	0	0
Enzootic pneumonia in swine	ELISA	**Swine**	0	0	0	0
	PCR Dubosson		0	0	0	0
Tularaemia	ID		0	0	0	0
Yersiniosis	ID	**Cat**	1	0	0	1
Campylobacteriosis	ID	**Diverse**	12	3	0	9
Antimicrobial resistance	ID, MIC	**Diverse**	3	0	0	3

3.2.4 Serotyping of *Salmonella* spp.

Table 8: Number of *Salmonella* spp. isolates for serotyping

Serovar	Animal	Number
S. Abony	Cattle	2
S. Abortusovis	Sheep	1
S. Adelaide	Zoo animal	1
S. Agona	Chicken	2
	Dog	1
S. Albany	Turkey	9
	Chicken	1
S. Bukavu	Reptil	1
	Zoo animal	3
S. Cardoner	Turkey	1
S. Chester	Reptil	1
S. Coeln	Chicken	2
S. Dublin	Cattle	2
S. Enteritidis	Cattle	4

Serovar	Animal	Number
S. Enteritidis	Chicken	19
	Dog	1
	Fox	1
	Guinea pig	1
	Horse	1
	Reptil	1
S. Gallinarum Biovar Pullorum	Chicken	1
S. Hadar	Cat	1
S. Infantis	Chicken	4
S. Kisarawe	Reptil	1
S. Livingstone	Chicken	3
S. Mbandaka	Chicken	1
S. Mikawasima	Cattle	2
S. Napoli	Chicken	2
	Cat	1
	Goat	1
S. Nigeria	Zoo animal	1
	Quail	2
S. Oranienburg	Reptil	1
S. Parathyphi B (d-Tartrat positiv; ehemals Java)	Dog	1
S. Reading	Cattle	1
S. Rissen	Pig	2
S. Sanga	Chicken	1
S. Schwarzengrund	Dog	1
S. Senftenberg	Turkey	2
	Reptil	2
S. Stourbridge	Chicken	2
S. Tennessee	Chicken	1
	Reptil	1
S. Typhimurium	Birds	2
	Cat	1
	Cattle	17
	Chicken	18
	Horse	6

Serovar	Animal	Number
S. Typhimurium	Donkey	1
	Dog	4
S. Typhimurium, monophasic variant (4,12:i:-)	Cat	2
	Cattle	2
	Chicken	2
S. Veneziana	Chicken	1
S. Welikade	Chicken	2
S. enterica subsp. *enterica* 13,23:i:-	Chicken	6
S. enterica subsp. *enterica* 47:z4,z23:- (monophasic)	Chicken	1
S. enterica subsp. diarizonae 61:k:1,5,7	Chicken	3
	Sheep	3
S. enterica subsp. *diarizonae* 38:z52:z53*	Bird	1
S. enterica subsp. *diarizonae* 48:z52:z*	Bird	1
S. enterica subsp. *diarizonae* 50:k:z*	Reptil	1
S. enterica subsp. *diarizonae* rough:z4,z23:-	Reptil	1
No *Salmonella*		1
	Total	**166**

* serotyping in human reference laboratory

3.2.5 Swine actinobacillosis: PCR based identification of *Actinobacillus pleuropneumoniae* by *apx* toxin gene typing and *cps2* gene detection

Table 9: Number of *Actinobacillus pleuropneumoniae* (APP) isolates for typing received from other laboratories

Biovar	**apx group**	**Serotype**	**Number**
Biovar I	apx group: I BD + II CA	7,12	22
Biovar I	apx group: I BD + II CA + III CA + BD *cps*2 gene positive	2	3
Biovar I	apx group: II CA + III CA + BD	3	3
Biovar II	apx group: I BD + II CA *cps*2 gene positive	2	7
Biovar I	Variant Serotype 3	-	3
No APP	-	-	0
		Total	**38**

3.2.6 Leptospirosis Diagnostics for Humans

Table 10: Number of samples investigated related to humane medicine

Zoonosis	**Agent**	**Method**	**Host**	**Total**	**Negative**	**Suspicious**	**Positive**
Leptospirosis	*L.* Australis	MAT	**Human**	1	1	0	0
	L. Autumnalis			1	1	0	0
	L. Bataviae			1	1	0	0
	L. Bratislava			1	1	0	0
	L. Canicola			1	0	1	0
	L. Celledoni			1	1	0	0
	L. Copenhageni			1	1	0	0
	L. Grippotyphosa			1	1	0	0
	L. Hardjo			1	1	0	0
	L. Hebdomalis			1	0	0	1
	L. Icterohaemo-rrhagiae			1	1	0	0
	L. Javanica			1	1	0	0
	L. Panama			1	1	0	0

Zoonosis	Agent	Method	Host	Total	Negative	Suspicious	Positive
Leptospirosis	*L.* Patoc	MAT	**Human**	1	1	0	0
	L. Pomona			1	1	0	0
	L. Pyrogenes			1	1	0	0
	L. Sejroe			1	1	0	0
	L. Shermani			1	1	0	0
	L. Tarassovi			1	1	0	0
	pathogene Leptospiren	PCR	**Human**	1	1	0	0

3.2.7 Organisation of Proficiency Testing or Surveys for approved laboratories

As Swiss national reference laboratory for a broad range of zoonotic or epizootic diseases and antimicrobial resistance the ZOBA is responsible for the diagnostic quality of the approved laboratories in Switzerland. For this purpose the ZOBA organized proficiency testings and surveys for these laboratories, which are mandatory for the approval by the Federal Food Safety and Veterinary Office. The conducted proficiency testing in 2022 are listed in Table 11.

Table 11: Survey for approved laboratories organised by the ZOBA in 2022

Target	Method	Number of samples	Number of laboratories
Coxelliosis	Real-timePCR	10	8
Brucellosis	ELISA	10	9

3.3 Research Activities

3.3.1 *Corynebacterium uberis* sp. nov. frequently isolated from bovine mastitis

Publication: Sonja Kittl, Eveline Studer, Isabelle Brodard, Andreas Thomann, Jörg Jores (2022) Syst Appl Microbiol. 45:126325, DOI: 10.1016/j.syapm.2022.126325

Collaborators: Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland.

Abstract: Several strains belonging to the genus *Corynebacterium*, but not to any described species of the genus were isolated from bovine mastitic milk samples over the past five years in the diagnostic unit of the University of Bern. Six of these strains (18M0132T, 17M2518, 18M0913, 19M0083, 20M1046 and 20M1090) that were phenotypically similar were further characterized genotypically. Gram-positive coryneform rods were catalase positive, facultative anaerobe and CAMP-test negative. Whole genome sequencing and subsequent phylogenetic analysis revealed their genome size to be 2.53 Mb and their G + C content to be between 65.4

and 65.5 mol%. Digital DNA-DNA hybridisation (dDDH) showed the highest similarity of only less than 20% with *Corynebacterium mastitidis* and *Corynebacterium frankenforstense*, which indicated that the isolates belong to an undescribed *Corynebacterium* species. This was confirmed by studying the average nucleotide identity (ANI) where the accepted species boundary is around 95% and which ranged between 70.3% and 74.9% with the most closely related species *C. mastitidis*. We established MALDI-TOF fingerprints of the species, which allows a clear separation from related species and can be used by other laboratories for diagnostic purposes. Based on our analyses we conclude that the selected strains belong to a previously undescribed species and propose the name *Corynebacterium uberis* sp. nov. The proposed type strain is 18M0132^T (=DSM111922^T, = CCOS 1972^T).

3.3.2 Quality of MALDI-TOF Mass Spectra in Routine Diagnostics: Results from an International External Quality Assessment including 36 Laboratories from 12 countries using 47 challenging bacterial strains

Publication: Cuénod A, Aerni M, Bagutti C, Bayraktar B, Boz ES, Carneiro CB, Casanova, C, Coste AT, Damborg P, van Dam D, Demirci M, Drevinek P, Dubuis O, Fernandez J, Greub G, Hrabak J, Yiğitler GH, Hurych J, Jensen TG, Jost G, Kampinga GA, Kittl S, Lammens C, Lang C, Lienhard R, Logan J, Maffioli C, Mareković I, Marschal M, Moran-Gilad J, Nolte O, Oberle M, Pedersen M, Pflüger V, Pranghofer S, Reichl J, Rentenaar RJ, Riat A, Rodríguez-Sánchez B, Schilt C, Schlotterbeck A-K, Schrenzel J, Troib S, Willems E, Wootton M, Ziegler D, Egli A. (2022) Clin Microbiol Infect. May 25:S1198-743X(22)00273-7. doi: 10.1016/j.cmi.2022.05.017.

Collaborators: Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland; Division of Clinical Bacteriology and Mycology, University Hospital Basel, Basel, Switzerland; Labor Team W, Goldach, Switzerland; State Laboratory Basel-Stadt, Basel, Switzerland; University of Health Sciences, Sisli Hamidiye Etfal Teaching and Research Hospital, Istanbul, Turkey; Department of Medical Microbiology, University of Health Sciences, Haydarpasa Numune Teaching and Research Hospital, Istanbul, Turkey; University Hospital Freiburg, Freiburg im Breisgau, Germany; Institute for Infectious Diseases, University of Bern, Bern, Switzerland; Institute of Microbiology, University Hospital Lausanne, Lausanne, Switzerland; Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark; Zuyderland MC, Sittard, the Netherlands; Department of Medical Microbiology, Kirklareli University, Kirklareli, Turkey; Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University and Motol University Hospital, Prague, Czech Republic; Viollier AG, Allschwil, Switzerland; Division of Laboratory Medicine, Laboratory of Bacteriology, University Hospital of Geneva, Geneva, Switzerland; Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Plzen, Czech Republic; Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark; Dianalabs, Geneva, Switzerland; Department of Medical Microbiology and Infection

Prevention, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; Department of Medical Microbiology, University of Antwerp, Belgium; ADMED Microbiologie, La Chaux de Fonds, Switzerland; Reference Services Division, UK Health Security Agency, London, United Kingdom; MCL Laboratories, Niederwangen, Switzerland; Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb, Zagreb, Croatia; Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany; School of Public Health, Ben Gurion University of the Negev and Soroka University Medical Center, Beer Sheva, Israel; Center for Laboratory Medicine, St. Gallen, Switzerland; Cantonal Hospital Aarau, Aarau, Switzerland; Department of Clinical Microbiology, Hvidovre Hospital, Hvidovre, Denmark; Mabritec AG, Riehen, Switzerland; Bioanalytica AG, Lucerne, Switzerland; Austrian Agency for Health and Food Safety, Vienna, Austria; UMC Utrecht, Utrecht, the Netherlands; Hospital General Universitario Gregorio Maranon, Madrid, Spain; Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland; Clinical Laboratory AZNikolaas, Sint-Niklaas, Belgium; University Hospital of Wales, Cardiff, United Kingdom; Eurofins Scientific AG, Schönenwerd, Switzerland; Applied Microbiology Research, Department of Biomedicine, University of Basel, Basel, Switzerland; Division of Clinical Bacteriology and Mycology, University Hospital Basel, Basel, Switzerland.

Abstract: **Objectives:** Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) is a widely used method for bacterial species identification. Incomplete databases and mass spectral quality (MSQ) still represent major challenges. Important proxies for MSQ are the number of detected marker masses, reproducibility, and measurement precision. We aimed to assess MSQs across diagnostic laboratories and the potential of simple workflow adaptations to improve it. **Methods:** For baseline MSQ assessment, 47 diverse bacterial strains, which are challenging to identify by MALDI-TOF MS, were routinely measured in 36 laboratories from 12 countries, and well-defined MSQ features were used. After an intervention consisting of detailed reported feedback and instructions on how to acquire MALDI-TOF mass spectra, measurements were repeated and MSQs were compared. **Results:** At baseline, we observed heterogeneous MSQ between the devices, considering the median number of marker masses detected (range = [2-25]), reproducibility between technical replicates (range = [55%-86%]), and measurement error (range = [147 parts per million (ppm)-588 ppm]). As a general trend, the spectral quality was improved after the intervention for devices, which yielded low MSQs in the baseline assessment as follows: for four out of five devices with a high measurement error, the measurement precision was improved (p-values <0.001, paired Wilcoxon test); for six out of ten devices, which detected a low number of marker masses, the number of detected marker masses increased (p-values <0.001, paired Wilcoxon test). **Discussion:** We have identified simple workflow adaptations, which, to some extent, improve MSQ of poorly performing devices and should be considered by laboratories yielding a low MSQ. Improving MALDI-TOF MSQ in routine diagnostics is

essential for increasing the resolution of bacterial identification by MALDI-TOF MS, which is dependent on the reproducible detection of marker masses. The heterogeneity identified in this external quality assessment (EQA) requires further study.

3.3.3 Prevalence and antimicrobial resistance of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) in Swiss sheep flocks

Publication: Weber M, Zanolari P, Ardüser F, Stucki D, Akarsu H, Overesch G (2022) Prev Vet Med. 2022 Sep;206:105697. doi: 10.1016/j.prevetmed.2022.105697.

Collaborators: Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland.

Abstract: Salmonella (S.) enterica subspecies *diarizonae* (IIIb) serovar 61:k:1,5,(7) (S. IIIb 61:k:1,5,(7)) is considered to be sheep-associated, as it can be found in the intestine, tonsils and nose of clinically healthy sheep, but it has also been described in separate clinical disorders in sheep. In particular, S. IIIb 61:k:1,5,(7) is described as the causative agent of chronic proliferative rhinitis (CPR) in sheep. In Switzerland, CPR in sheep due to S. IIIb 61:k:1,5,(7) was first described in 2017 in a flock of Texel sheep. Therefore, we assessed the prevalence of S. IIIb 61:k:1,5,(7) within the Swiss sheep population using a representative sampling strategy. From May 2017 to June 2018 a total of 681 nasal swabs from individual clinically healthy sheep of 141 different flocks throughout Switzerland were taken. Swabs were analysed by selective enrichment for the presence of S. IIIb 61:k:1,5,(7). Additionally, antimicrobial resistance of the isolates was determined by broth microdilution. A total of 146 out of 681 nasal swabs tested positive for S. IIIb 61:k:1,5,(7), which corresponds to a prevalence on animal level of 21% (95%CI 18%-25%). In 73 out of 141 flocks tested, at least one sheep tested positive for S. IIIb 61:k:1.5,(7), resulting in a minimal prevalence on flock level of 52% (95%CI 43%-60%). Positive flocks were found in all cantons except the canton of Jura. Adults were significantly more affected than sheep under one year/lambs and positive sheep were found in several breeds. No microbiologically resistant isolates were detected, except for one isolate showing resistance against ampicillin. Because of its widespread occurrence in the Swiss sheep population, further research should focus on the pathogenic impact of S. IIIb 61:k:1,5,(7) on the health status of sheep.

3.3.4 Bayesian latent class models to determine diagnostic sensitivities and specificities of two point of care rapid tests (Selma plus, Dipslide) for the detection of *Streptococcus uberis* associated with mastitis in dairy cows

Publication: Rediger D, Butty MA, Kittl S, Bodmer M, Hartnack S. (2022) Front Vet Sci. 2022 Dec 13;9:1062056. doi: 10.3389/fvets.2022.1062056.

Collaborators: Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, CH-3012 Bern, Switzerland. Section of Veterinary Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.

Abstract: **Introduction:** Development and validations of accurate mastitis diagnostics are crucial to make timely and evidence-based decisions on mastitis therapy in order to reduce its impact on productivity, animal welfare and practicing the prudent use of antimicrobials on dairy farms. **Methods:** The objectives of this study were to assess the agreement between test results from reference laboratory and two point of care tests (Selma plus, Dipslide) and to estimate the test accuracies with Bayesian latent class models (BLCMs). In total of 509 single quarter milk samples from cows with mastitis were included in the study. **Results**: Among all analyzed mastitis pathogens, Streptococcus spp. was detected in up to one third of all analyzed samples and for Selma all Streptococcus samples were considered as Streptococcus uberis. The agreement (κ) when comparing two tests varied greatly depending on the bacteria, ranging from no agreement to good agreement (κ = negative to 0.86) depending on the prevalence of identified pathogens. Based on BLCMs to assess diagnostic test accuracies for the pathogen Streptococcus uberis, posterior sensitivities of 76, 71, and 64% for Selma plus, Dipslide and laboratory standard culture and specificities of 93%, 98% for Selma and Dipslide, respectively, were obtained. **Discussion:** The two point of care rapid culture systems Dipslide and Selma plus plate can provide important preliminary pathogen identification for targeted mastitis therapy, especially when general information about growth and a rough classification of the bacteria into groups have an impact on treatment strategy. The two evaluated rapid culture systems, Dipslide and Selma plus plate, show good test accuracies for Streptococcus uberis at least at genus level. Therefore, using these tests may contribute to prudent use of antibiotics.

4 Teaching Obligations

4.1 Bacteriology Lecture Series

General Bacteriology and Mycology:	26 x 45 min
Clinical Bacteriology and Mycology:	26 x 45 min

4.2 Organ Specific Lectures

Blood/Laboratory/Immune system:	1 x 45 min
Skin and Thermoregulation:	1 x 45 min
Renal system:	1 x 45 min
Respiratory system:	1 x 45 min

4.3 Clinical Signs

Coughing / Dyspnoe pig	4 x 45 min

4.4 Hands on Courses

General Practical Course in Bacteriology:	48 x 45 min
Practical Course for Pathobiology Major students:	58 x 45 min

4.5 Paraclinic Days

Attachment to IVB researchers for a day (8 h):	12 students

5 Publications

5.1 Peer-Reviewed Publications

1. Akarsu H, Liljander A, Younan M, Brodard I, Overesch G, Glucks I, Labroussaa F, Kuhnert P, Perreten V, Monecke S, Drexler JF, Corman VM, Falquet L, Jores J. 2022. Genomic Characterization and Antimicrobial Susceptibility of Dromedary-Associated *Staphylococcaceae* from the Horn of Africa. Appl Environ Microbiol 88:e0114622.
2. Arnold M, Schmitt S, Collaud A, Rossano A, Hubschke E, Zeeh F, Nathues H, Perreten V. 2022. Distribution, genetic heterogeneity, and antimicrobial susceptibility of *Brachyspira pilosicoli* in Swiss pig herds. Vet Microbiol 269:109421.
3. Becker J, Fernandez JE, Rossano A, Meylan M, Perreten V. 2022. Clonal dissemination of MDR *Pasteurella multocida* ST79 in a small Swiss veal calf farm with high use of antibiotics. J Antimicrob Chemother 77:2886-2888.
4. Becker J, Perreten V, Schupbach-Regula G, Stucki D, Steiner A, Meylan M. 2022. Associations of antimicrobial use with antimicrobial susceptibility at the calf level in bacteria isolated from the respiratory and digestive tracts of veal calves before slaughter. J Antimicrob Chemother 77:2859-2866.
5. Becker J, Perreten V, Steiner A, Stucki D, Schupbach-Regula G, Collaud A, Rossano A, Wuthrich D, Muff-Hausherr A, Meylan M. 2022. Antimicrobial susceptibility in *E. coli* and *Pasteurellaceae* at the beginning and at the end of the fattening process in veal calves: Comparing 'outdoor veal calf' and conventional operations. Vet Microbiol 269:109419.
6. Beer J, Crotta S, Breithaupt A, Ohnemus A, Becker J, Sachs B, Kern L, Llorian M, Ebert N, Labroussaa F, Nhu Thao TT, Trueeb BS, Jores J, Thiel V, Beer M, Fuchs J, Kochs G, Wack A, Schwemmle M, Schnepf D. 2022. Impaired immune response drives age-dependent severity of COVID-19. J Exp Med 219:e20220621.
7. Brown A, Rodriguez V, Pfister J, Perreten V, Neumann P, Retschnig G. 2022. The dose makes the poison: feeding of antibiotic-treated winter honey bees, *Apis mellifera*, with probiotics and b-vitamins. Apidologie 53:19.
8. Budnik M, Struck AK, Storms J, Wirth A, Jores J, Kuhnert P, Distl O. 2022. Serological Diversity of *Dichelobacter nodosus* in German Sheep Flocks. Animals (Basel) 12:753.
9. Campos-Madueno EI, Moser AI, Jost G, Maffioli C, Bodmer T, Perreten V, Endimiani A. 2022. Carbapenemase-producing *Klebsiella pneumoniae* strains in Switzerland:

human and non-human settings may share high-risk clones. J Glob Antimicrob Resist 28:206-215.

10. Cottagnoud P, Sprenker F, Cottagnoud M, Collaud A, Ashkbus R, Perreten V. 2022. Addition of daptomycin to levofloxacin increased the efficacy of levofloxacin monotherapy against a methicillin-susceptible *Staphylococcus aureus* strain in experimental meningitis and prevented development of resistance *in vitro*. J Med Microbiol 71:DOI: 10.1099/jmm.0.001497.
11. Cuenod A, Aerni M, Bagutti C, Bayraktar B, Boz ES, Carneiro CB, Casanova C, Coste AT, Damborg P, van Dam DW, Demirci M, Drevinek P, Dubuis O, Fernandez J, Greub G, Hrabak J, Hurkal Yigitler G, Hurych J, Jensen TG, Jost G, Kampinga GA, Kittl S, Lammens C, Lang C, Lienhard R, Logan J, Maffioli C, Marekovic I, Marschal M, Moran-Gilad J, Nolte O, Oberle M, Pedersen M, Pfluger V, Pranghofer S, Reichl J, Rentenaar RJ, Riat A, Rodriguez-Sanchez B, Schilt C, Schlotterbeck AK, Schrenzel J, Troib S, Willems E, Wootton M, Ziegler D, Egli A, group Es. 2022. Quality of MALDI-TOF mass spectra in routine diagnostics: results from an international external quality assessment including 36 laboratories from 12 countries using 47 challenging bacterial strains. Clin Microbiol Infect doi:10.1016/j.cmi.2022.05.017.
12. Demoulins T, Brugger M, Zumkehr B, Oliveira Esteves BI, Ruggli N, Alves MP. 2022. Multiparameter flow cytometry assay to analyze the pulmonary T cell profiles in the ovine model of respiratory syncytial virus infection. STAR Protoc 3:101688.
13. Dona V, Ramette A, Perreten V. 2022. Comparative genomics of 26 complete circular genomes of 18 different serotypes of *Actinobacillus pleuropneumoniae*. Microb Genom 8:000776.
14. Feitosa-Junior OR, Souza APS, Zaini PA, Baccari C, Ionescu M, Pierry PM, Uceda-Campos G, Labroussaa F, Almeida RPP, Lindow SE, da Silva AM. 2022. The XadA Trimeric Autotransporter Adhesins in *Xylella fastidiosa* Differentially Contribute to Cell Aggregation, Biofilm Formation, Insect Transmission and Virulence to Plants. Mol Plant Microbe Interact 35:857-866.
15. Fernandez JE, Seth-Smith HMB, Nordmann P, Egli A, Endimiani A, Perreten V. 2022. Intra- and Interspecies Spread of a Novel Conjugative Multidrug Resistance *IncC* Plasmid Coharboring *bla*$_{\text{OXA-181}}$ and *armA* in a Cystic Fibrosis Patient. Microbiol Spectr 10:e0312122.

16. Findlay J, Perreten V, Poirel L, Nordmann P. 2022. Molecular analysis of OXA-48-producing *Escherichia coli* in Switzerland from 2019 to 2020. Eur J Clin Microbiol Infect Dis 41:1355-1360.
17. Garcia-Martin AB, Roder T, Schmitt S, Zeeh F, Bruggmann R, Perreten V. 2022. Whole-genome analyses reveal a novel prophage and cgSNPs-derived sublineages of *Brachyspira hyodysenteriae* ST196. BMC Genomics 23:131.
18. Hurni JI, Kaiser-Thom S, Gerber V, Keller JE, Collaud A, Fernandez J, Schwendener S, Perreten V. 2022. Prevalence and whole genome-based phylogenetic, virulence and antibiotic -resistance characteristics of nasal -*Staphylococcus aureus* in healthy Swiss horses. Schweiz Arch Tierheilkd 164:499-512.
19. Kaiser-Thom S, Gerber V, Collaud A, Hurni J, Perreten V. 2022. Prevalence and WGS-based characteristics of *Staphylococcus aureus* in the nasal mucosa and pastern of horses with equine pastern dermatitis. BMC Vet Res 18:79.
20. Keller JE, Schwendener S, Neuenschwander J, Overesch G, Perreten V. 2022. Prevalence and characterization of -methicillin-resistant *Macrococcus* spp. in food producing animals and meat in Switzerland in 2019. Schweiz Arch Tierheilkd 164:153-164.
21. Keller JE, Schwendener S, Overesch G, Perreten V. 2022. *Macrococcus armenti* sp. nov., a novel bacterium isolated from the skin and nasal cavities of healthy pigs and calves. Int J Syst Evol Microbiol 72:005245.
22. Kittl S, Studer E, Brodard I, Thomann A, Jores J. 2022. *Corynebacterium uberis* sp. nov. frequently isolated from bovine mastitis. Syst Appl Microbiol 45:126325.
23. Kuhnert P, Brodard I, Bock S, Hemphill A, Akarsu H, Engelhardt A, Kutzer P. 2022. *Wielerella bovis* gen. nov., sp. nov. a member of the family *Neisseriaceae* associated with bovine endocarditis. Int J Syst Evol Microbiol 72:005387.
24. Larsen J, Raisen CL, Ba X, Sadgrove NJ, Padilla-Gonzalez GF, Simmonds MSJ, Loncaric I, Kerschner H, Apfalter P, Hartl R, Deplano A, Vandendriessche S, Cerna Bolfikova B, Hulva P, Arendrup MC, Hare RK, Barnadas C, Stegger M, Sieber RN, Skov RL, Petersen A, Angen O, Rasmussen SL, Espinosa-Gongora C, Aarestrup FM, Lindholm LJ, Nykasenoja SM, Laurent F, Becker K, Walther B, Kehrenberg C, Cuny C, Layer F, Werner G, Witte W, Stamm I, Moroni P, Jorgensen HJ, de Lencastre H, Cercenado E, Garcia-Garrote F, Borjesson S, Haeggman S, Perreten V, Teale CJ, Waller AS, Pichon B, Curran MD, Ellington MJ, Welch JJ, et al. 2022. Emergence of methicillin resistance predates the clinical use of antibiotics. Nature 602:135-141.

25. Mansour M, Giudice E, Xu X, Akarsu H, Bordes P, Guillet V, Bigot DJ, Slama N, D'Urso G, Chat S, Redder P, Falquet L, Mourey L, Gillet R, Genevaux P. 2022. Substrate recognition and cryo-EM structure of the ribosome-bound TAC toxin of *Mycobacterium tuberculosis*. Nat Commun 13:2641.
26. Marchionatti E, Perreten V. 2022. Whole-Genome Sequences of Antibiotic-Resistant *Trueperella pyogenes* Isolates from Surgical Site Infections in Dairy Cows in Switzerland. Microbiol Resour Announc 11:e0086522.
27. Marenzoni ML, Stefanetti V, Del Rossi E, Zicavo A, Scuota S, Origgi FC, Deli G, Corti C, Trabalza Marinucci M, Olivieri O. 2022. Detection of Testudinid alphaherpesvirus, *Chlamydia* spp., *Mycoplasma* spp., and *Salmonella* spp. in free-ranging and rescued Italian *Testudo hermanni hermanni*. Vet Ital 58:25-34.
28. Menezes J, Moreira da Silva J, Frosini SM, Loeffler A, Weese S, Perreten V, Schwarz S, Telo da Gama L, Amaral AJ, Pomba C. 2022. *mcr-1* colistin resistance gene sharing between *Escherichia coli* from cohabiting dogs and humans, Lisbon, Portugal, 2018 to 2020. Euro Surveill 27:2101144.
29. Moser AI, Campos-Madueno EI, Perreten V, Endimiani A. 2022. Genome stability during serial subculturing in hyperepidemic multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*. J Glob Antimicrob Resist 31:152-161.
30. Rediger D, Butty MA, Kittl S, Bodmer M, Hartnack S. 2022. Bayesian latent class models to determine diagnostic sensitivities and specificities of two point of care rapid tests (Selma plus, Dipslide) for the detection of *Streptococcus uberis* associated with mastitis in dairy cows. Front Vet Sci 9:1062056.
31. Scalisi N, Kuhnert P, Amado MEV, Overesch G, Stark KDC, Ruggli N, Jores J. 2022. Seroprevalence of *Mycoplasma hyopneumoniae* in sows fifteen years after implementation of a control programme for enzootic pneumonia in Switzerland. Vet Microbiol 270:109455.
32. Schnyder A, Eberl L, Agnoli K. 2022. Investigating the Biocontrol Potential of the Natural Microbiota of the Apple Blossom. Microorganisms 10:2480.
33. Schwendener S, Perreten V. 2022. The *bla* and *mec* families of beta-lactam resistance genes in the genera *Macrococcus*, *Mammaliicoccus* and *Staphylococcus*: an in-depth analysis with emphasis on *Macrococcus*. J Antimicrob Chemother 77:1796-1827.
34. Storms J, Wirth A, Vasiliadis D, Jores J, Kuhnert P, Distl O. 2022. Risk factors associated with the infection of sheep with *Dichelobacter nodosus*. Sci Rep 12:10032.

35. Talenton V, Baby V, Gourgues G, Mouden C, Claverol S, Vashee S, Blanchard A, Labroussaa F, Jores J, Arfi Y, Sirand-Pugnet P, Lartigue C. 2022. Genome Engineering of the Fast-Growing *Mycoplasma feriruminatoris* toward a Live Vaccine Chassis. ACS Synth Biol 11:1919-1930.
36. Ulrich L, Halwe NJ, Taddeo A, Ebert N, Schon J, Devisme C, Trueb BS, Hoffmann B, Wider M, Fan X, Bekliz M, Essaidi-Laziosi M, Schmidt ML, Niemeyer D, Corman VM, Kraft A, Godel A, Laloli L, Kelly JN, Calderon BM, Breithaupt A, Wylezich C, Berenguer Veiga I, Gultom M, Osman S, Zhou B, Adea K, Meyer B, Eberhardt CS, Thomann L, Gsell M, Labroussaa F, Jores J, Summerfield A, Drosten C, Eckerle IA, Wentworth DE, Dijkman R, Hoffmann D, Thiel V, Beer M, Benarafa C. 2022. Enhanced fitness of SARS-CoV-2 variant of concern Alpha but not Beta. Nature 602:307-313.
37. Wambui J, Stevens MJA, Sieber S, Cernela N, Perreten V, Stephan R. 2022. Targeted Genome Mining Reveals the Psychrophilic *Clostridium estertheticum* Complex as a Potential Source for Novel Bacteriocins, Including Cesin A and Estercticin A. Front Microbiol 12:801467.
38. Weber M, Zanolari P, Arduser F, Stucki D, Akarsu H, Overesch G. 2022. Prevalence and antimicrobial resistance of *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,(7) in Swiss sheep flocks. Prev Vet Med 206:105697.

5.2 Book Chapters

- Chapter 14: MALDI-TOF MS Analysis for Identification of Veterinary Pathogens from Companion Animals and Livestock Species, Dorina Timofte, Gudrun Overesch and Joachim Spergser in Microbiological Identification using MALDI-TOF and Tandem Mass Spectrometry: Industrial and Environmental Applications. Haroun N. Shah (Editor), Saheer E. Gharbia (Editor), Ajit Shah (Editor), Erika Tranfield (Editor), Clive Thompson (Editor) Wiley Press May 2023; ISBN: 978-1-119-81405-4

5.3 Other Publications

- «Molecular surveillance of pathogens in Switzerland – Focus: SARS-CoV-2 and its variants» by Aitana Neves, Dominique Blanc, Gilbert Greub, Hans H. Hirsch, Michael Huber, Laurent Kaiser, Stephen L. Leib, Vincent Perreten, Jacques Schrenzel, Roger Stephan, Reinhard Zbinden, Adrian Egli, PIPETTE – SWISS LABORATORY MEDICINE | WWW. SULM.CH Pipette No. 3, Page 9-11 | June, 2022

- Federal Office of Public Health and Federal Food Safety and Veterinary Office. Swiss Antibiotic Resistance Report 2022. Usage of Antibiotics and Occurrence of Antibiotic Resistance in Switzerland. November 2022.
- New kid on the block: Corynebacterium uberis. M^2-magazine (Magazine on Mastitis and Milk quality for the dairy professional). September 2022.

5.4 Press Releases and Broadcasting

- Les hérissons portent des bactéries résistantes aux antibiotiques https://www.24heures.ch/les-herissons-portent-des-bacteries-resistantes-aux-antibiotiques-890256785168 07.01.2022, 18h08
- Bactéries résistantes aux antibiotiques sur les hérissons https://www.lemanbleu.ch/fr/Actualite/Economie/Bacteries-resistantes-aux-antibiotiques-sur-les-herissons.html 07.01.2022, 15h48
- Bactéries résistantes aux antibiotiques sur les hérissons https://www.swissinfo.ch/fre/bact%C3%A9ries-r%C3%A9sistantes-aux-antibiotiques-sur-les-h%C3%A9rissons/47245280 07 janvier 2022 - 15:48
- les hérissons portent des bactéries résistantes aux antibiotiques - https://swiss.dayfr.com/local/amp/11666 Saturday 08th January 2022 05:16 AM
- Igel beherbergen seit 200 Jahren antibiotikaresistente Bakterien https://www.fm1today.ch/schweiz/igel-beherbergen-seit-200-jahren-antibiotikaresistente-bakterien-144980489 - 6. Januar 2022 17:18
- Ihre Haut ist wertvoll - Igel beherbergen seit 200 Jahren antibiotikaresistente Bakterien https://www.blick.ch/schweiz/ihre-haut-ist-wertvoll-igel-beherbergen-seit-200-jahren-antibiotikaresistente-bakterien-id17121528.html 05.01.2022 um 17:04 Uhr
- Une bactérie résistante aux antibiotiques découverte sur les hérissons https://www.rts.ch/info/sciences-tech/12771357-une-bacterie-resistante-aux-antibiotiques-decouverte-sur-les-herissons.html 17:41 Modifié vendredi à 20:44
- Igel beherbergen seit 200 Jahren antibiotikaresistente Bakterien https://www.bielertagblatt.ch/igel-beherbergen-seit-200-jahren-antibiotikaresistente-bakterien 05.01.2022, 17:00

6 Graduations, Promotions, and Visting Scientists

6.1 American College of Veterinary Microbiology (ACVM)

- N/A

6.2 European College of Veterinary Microbiology (ECVM)

- Natascha Gross started her residency programme in 2022.

6.3 Promotions

Scientist to principal scientist: Fabien Labroussaa

Area of specialization: Synthetic Genomics and Host-Pathogen Interactions

6.4 PhD Degrees

Name of student: Paul Ssajjakambwe (Makerere University, Uganda)

Title of Thesis: Application of synthetic genomics to generate *Mycoplasma mycoides* mutants to decipher host-pathogen interactions

Supervisors: Robert Tweyongyere (Makerere University, Uganda), Enock Matovu (Makerere University, Uganda), Jörg Jores and Elise Schieck (International Livestock Research Institute, Kenya)

Abstract: The motivation for this study was to have an in-depth understanding of the biology of *Mycoplasma mycoides* (*Mm*) cluster organisms that is hitherto deficient. The main objective was to apply transposon mutagenesis and synthetic biology technologies to decipher host pathogen interactions. Specifically, a cluster of 68 genes were deleted, which are know to contribute to the virulence of Mycoplamas. These included those that contribute to Glycerol uptake, a key metabolite for these bacteria, transmembrane carrier proteins among others. For transposon-based mutagenesis a plasmid (pMT85-tetM-PRS313-LacZ) was introduced into *Mycoplasma mycoides* cluster strains by electroporation and chemical methods. The developed transposon mutagenesis libraries these pathogens affecting livestock, the first of its kind in Africa. However, the libraries generated among the *Mycoplasma mycoides* subsp. *mycoides* and *Mycoplasma mycoides* subsp. *capri* were not stable over time since their recovery after storage was not good as compared to those of *Mycoplasma capricolum* subsp. *capricolum*. Subsequently, genes earlier on known to play a role in the virulence of Mycoplasma such as (GlpF, GlpK, GlpO, gtsA, gtsB, gtsC & gtsD) were deleted using the Tandem Repeat Endonuclease cleavage (TREC) method. The mentioned genes were selected based on their functions from earlier studies and literature. This was under the assumption that the generated mutant (GM12::YCpMmyc1.1-Δ68) would turn out to be less virulent. To investigate this

postulation, an *in-vivo* experiment model was advanced as proof of principle. Using a *Mycoplasma mycoides* subsp. *capri* (*Mmc*) strain, a known goat pathogen, goats were each infected with 10^9 cfus live bacteria in two groups as follows; the first group (n=8) received the wild type strain *Mmc* GM12 whereas the second (n=6) received the presumably attenuated strain, GM12::YCpMmyc1.1-Δ68). All animals that received the wild type strain developed clinical disease characterized by pneumonia, coughing, a high fever (an average of 41^{o}C), inappetence and later anorexia. In addition, all candidates in this group never lived past day 5 post infection (pi). In comparison, all animals that received the mutant strain, though developed mild clinical disease initially characterized by slight depression, just after inoculation and recovered, reverted to normal behavior, living up-to day 28 pi, the intended end time for the experiment. The Karplan Meyer survival curve exhibited a clear distinction between the two experimental groups (*p-value* < 0.001). Further analysis of the mutant GM12::YCpMmyc1.1-Δ68 revealed that it had completely lost its immune evasion mechanism of Immunoglobulin G cleavage a trait otherwise retained in the parent strain. In addition, the mutant strain lost its ability to produce Hydrogen peroxide in the presence of glycerol *in-vitro* as indicated in the Hydrogen peroxide assay. This is the first-time transposon mutant libraries are generated in Africa among Mycoplasma pathogens that affect livestock, invaluable tools for in-depth studies. Proof of the true virulence factors in *Mmc* in both *in-vitro* and *in-vivo* experiments was demonstrated. These approaches are a gateway to additional research that shall contribute to improving the existing vaccine(s) or better still, new vaccine or drug candidates developed for Mycoplasmas in the *Mm* cluster.

Name of student: Ana Belén García-Martín

Title of Thesis: Antimicrobial resistance and molecular typing of *Brachyspira hyodysenteriae*: from traditional techniques to high-throughput sequencing.

Supervisor: Vincent Perreten

Abstract: Antimicrobial resistance is a problem of global concern that requires the fast identification of the molecular mechanisms underlying, as well as their spread, in order to treat and control important bacterial infectious diseases. Whole-genome sequencing has revolutionized the way in which antimicrobial resistance mechanisms have been investigated in the last decades. The advent of high-throughput sequencing platforms, especially those enabling long reads sequencing, has facilitated the reconstruction of complete genomes of bacterial pathogens relevant for human and veterinary medicine. Therefore, the availability of the complete genome of many different bacterial species has helped to identify novel

mechanisms underlying acquired antimicrobial resistance, also allowing to determine their exact location in the genome. An important swine pathogen that causes a severe inflammatory intestinal disease, i.e., swine dysentery, that can lead to pig death is *Brachyspira hyodysenteriae*. Despite that this pathogen is of worldwide concern, at the time of starting this doctoral thesis only two complete genomes belonging to the *B. hyodysenteriae* strain WA1 and the type strain B-78T were published. Furthermore, although the negative impact of *Brachyspira hyodysenteriae* on Swiss pig production was recognized in 2008, neither antimicrobial resistance mechanisms nor whole-genome sequencing-based studies have been carried out. Therefore, this doctoral thesis aimed to contribute to the *Brachyspira hyodysenteriae* field with fresh insights obtained from investigations based on high-throughput whole-genome sequencing technologies combined with traditional laboratory techniques. Different aspects related to genetic diversity, genomic structure, epidemiology and antimicrobial resistance mechanisms in *Brachyspira hyodysenteriae* have been covered in this doctoral thesis. The results presented in this dissertation provide not only relevant information of local interest but also of global interest, and open new lines of research.

6.5 Dr. vet. med. Degrees

Name of student: Nadia Scalisi

Title of Thesis: Seroprevalence of *Mycoplasma hyopneumoniae* in sows fifteen years after implementation of a control programme for enzootic pneumonia in Switzerland

Supervisor: Jörg Jores

Abstract: *Mycoplasma hyopneumoniae* is the etiological agent of enzootic pneumonia (EP), an economically important chronic respiratory disease in pigs. *M. hyopneumoniae* impacts the mucociliary clearance system by disrupting the cilia and modulates the immune response, resulting in intermittent dry non-productive cough. For progressive control of EP in Switzerland, a corresponding programme was fully implemented in 2004. It is based on total depopulation strategies of affected fattening farms as well as partial depopulation in breeding farms. Surveillance of EP status in Switzerland is mainly based on real-time PCR on nasal swabs from coughing animals or suspicious lungs and thereby sporadic cases are still observed every year. In order to get information on the seroprevalence, serum samples of 5021 sows from 968 farms collected in 2018 at eight different slaughterhouses were analyzed for the presence of *M. hyopneumoniae*-specific antibodies using a commercial ELISA kit. The overall seroprevalence was low with 0.98% of sows tested positive and these seropositive animals could be allocated to 3.92% of farms tested. Most seropositive farms presented weakly positive singleton reactors and only one farm showed several strongly seropositive animals. In conclusion, the serological status mirrors the successful progressive control of *M. hyopneumoniae* in the Swiss domestic pig population over the years. The current study

underlines the added value of serological testing in the surveillance of EP in Switzerland and confirms the sustained benefit of strategic control programmes.

Name of student: Milena Tresch

Title of Thesis: Surface expression of heterologous antigens in two *Mycoplasma* species

Supervisors: Fabien Labroussaa and Jörg Jores

Abstract: Control of contagious caprine pleuropneumonia (CCPP) relies on a bacterin, conferring immunity for up to one year. Bacterin production is cumbersome due to the fastidious nature and long division time of *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*). The development of a live vaccine CCPP, based on a fast-growing *Mycoplasma* vaccine chassis expressing heterologous antigens offers the possibility to be applied into the respiratory tract and to induce an immune response at the target tissue of the respiratory pathogens. This work explores the possibility of surface expression of heterologous antigens in fast-growing mycoplasmas. Using the reverse vaccinology pipeline ReVac, candidate vaccine antigens of *Mccp* were identified and the 6 antigens with the highest scores were used for subsequent work. The antigen-encoding genes were cloned in replicative plasmids and successfully expressed in *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma feriruminatoris*. Triton X-114 partitioning confirmed the membrane-associated location of these recombinant *Mccp* antigens. IN a next step we wanted to express heterologous viral proteins at the *Mycoplasma* surface. Therefore, we fused viral genes to either lipoprotein anchor- or transmembrane domain-encoding gene fragments. After transformation, the two viral proteins tested, namely M2e and hemagglutinin of Human Influenza virus A, were successfully expressed at the mycoplasma surface as shown by Triton X-114 partitioning and immunoblots. This work demonstrates the ability to direct heterologous protein expression via fusion to gene fragments encoding transmembrane domains or lipoprotein signal sequences at the membrane of *M. capricolum* subsp. *capricolum*.

Name of student: Jennifer Eleonora Keller

Title of Thesis: Methicillin-resistant *Macrococcus* spp. in food producing animals in Switzerland: prevalence, antimicrobial profiles and description of a novel species.

Supervisor: Vincent Perreten

Abstract: Prevalence of methicillin-resistant *Macrococcus* spp. in food producing calves and pigs in 2019 was determined with strains isolated within the framework of the national monitoring of methicillin-resistant *Staphylococcus aureus* (MRSA). The isolates were submitted to antimicrobial susceptibility testing and to molecular techniques for the

identification of antimicrobial resistance genes. Methicillin-resistant *Macrococcus* spp. were isolated in 40 of 299 nasal swabs from calves representing a prevalence of 13.38% (95% CI, 9.98% – 17.70%), and in four of 303 nasal swabs from pigs [1.32% (95% CI, 0.36% – 3.35%)]. One of 311 samples of Swiss pork meat contained a *Macrococcus* sp. [0.32% (95% CI, 0.01% – 1.78%)], and four of 309 beef meat samples (260 domestic and 49 imported) contained *M. caseolyticus* [1.29% (95% CI, 0.35% – 3.28%)]. Three strains could not be identified to the species level nor by MALDI-TOF MS neighter by *hsp60* and 16S-rDNA gene analysis. The MALDI-TOF MS spectral profile and 16S-rDNA gene sequence analysis of two strains isolated from calves showed that they belonged to the same species as a *Macrococcus* sp. strain isolated from a pig in 2017 isolated within the same framework. Strains further isolated in 2021 exhibited the same spectral profile and 16S-rDNA gene sequence. Based on genotypic and chemotaxonomic characteristics, these strains revealed to represent a novel species of the genus *Macrococcus*, for which we proposed the name *Macrococcus armenti* sp. nov.

6.6 Master Degrees

Name of student: Joel Immanuel Hurni (Master thesis in Veterinary Medicine)

Title of Thesis: Prevalence and nasal carriage of *Staphylococccus aureus* in Swiss healthy horses and resistance to antibiotics

Supervisor: Vincent Perreten

Name of student: Elio M. Elezović (Master thesis in Veterinary Medicine)

Title of Thesis: Carbapenemase producing *Escherichia coli* in Switzerland and ST410 around the world: an analysis of resistances, resistance genes and their distribution in humans, animals, and the environment.

Supervisor: Vincent Perreten

Name of student: Tatiana Zingre (Master thesis in Veterinary Medicine)

Title of Thesis: *Treponema* species within interdigital cleft lesions of wisent (European bison, *Bison bonasus*): a scanning and transmission electron microscopic study.

Supervisors: Peter Kuhnert and Sabine Kässmeyer

Name of student: Jil Neuenschwander (Master thesis in Molecular Life Sciences)

Title of Thesis: Characterization of the γ-haemolysin genes *hlgC* and *hlgB* of *Macrococcus canis* and the δ-toxin gene *hld* of *Macrococcus brunensis* and other *Macrococcus* species.

Supervisor: Sybille Schwendener and Vincent Perreten

6.7 Visiting Scientists and Guests

Prof Dr Pattrarat Chanchaithong. Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. Visiting Professor by Vincent Perreten "Whole genome sequencing and analysis of carbapenem-resistant *Escherichia coli* and *Acinetobacter baumannii* from companion animals of Thailand". February – August 2022

Prof Dr Eric Oswald, Bactériologie-Hygiène, CHU de Toulouse, France Toulouse (France), Seminar title: HlyF a fake Hemolysin but a real virulence factor: from sepsis to vaccination, 3rd March 2022

Prof Dr Michael Hust, Technical University of Braunschweig, Institute of Biochemistry and Biotechnology, (Germany), Seminar title: "Fighting infectious diseases with recombinant antibodies", 21st April 2022

Dr hc Claudine Andre, Belgian conservationist & founder of the Congolese sanctuary "Lola ya bonobo" (Democratic Republic of Congo), Doctor honoris causa of the University of Bern 2020, Seminar title: "Bonobo, a fascinating species in danger", 29th April 2022

Prof Christian Menge, Head of Institute of Molecular Pathogenesis at the FLI - Federal Research Institute for Animal Health, Jena (Germany), Seminar title: "Assessing the immunogenicity, safety, efficacy and interference with diagnostic tests of genetically modified BCG in a goat model of pulmonary *Mycobacterium bovis* infection", 5th May 2022

Prof Dr Laure Béven, University of Bordeaux, Deputy Director UMR 1332 BFP, INRAE Bordeaux, Bordeau (France), Seminar title: "Programming and tuning of shape and motility in minimal cells", 19th May 2022

Prof Dr Hubert Hilbi, Institute of Medical Microbiology, University of Zürich, Zürich (Switzerland), Seminar title: "*Legionella pneumophila* - a copycat eukaryote", 9th June 2022

Prof Dr Ottmar Distl, Chiar of Institute of Animal Breeding and Genetics, University of Veterinary Medicine Hannover (Foundation), Hannover (Germany), Seminar title: "MORes-project on ovine footrot in Germany - epidemiology, genetics and genomics", 22nd September 2022

Prof Dr Bernd Lepenies, Chair of Institute for Immunology, University of Veterinary Medicine Hannover (Foundation), Hannover (Germany), Seminar title: "The role of C-type lectin receptors in pathogen recognition and infectious diseases", 27th October 2022

Prof Dr Reinhard Straubinger, Chair of Bacteriology and Mycology, Faculty of Veterinary Medicine, Institute for Infectious Diseases and Zoonosis, LMU-Ludwig-Maximilians-University Munich, Munich (Germany), Seminar Title: "Next-Generation Sequencing in the Context of Lyme-Borreliosis", 10th November 2022

7 Scientific Meetings Organized, Keynotes given and Grants Awarded

7.1 Scientific Meetings Organized by IVB Staff

- Gudrun Overesch, Leader of Working group 2 of the COST Action CA18217 Title: European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT), Working groups and Management Committee Meeting, School of Veterinary Medicine, Aristotles University of Thessaloniki, Greece, 12-13 May 2022

7.2 Keynote/Invited Lectures Given by IVB Staff

- Joerg Jores, Title: A yeast-based synthetic genomics platform to reconstruct and edit *Mycoplasma* and viral genomes, VIDO Distinguished Seminar Series, March 30, Saskatoon (Canada), Invited Talk, online
- Joerg Jores, Title: Contagious bovine pleuropneumonia: current vaccines and future perspectives, International Veterinary Vaccinology Network (IVVN) and the United States Animal Vaccine Research Coordination Network (USAVRCN) webinar "Bovine *Mycoplasma* Vaccines and Immune Responses", March 23, Invited Talk, online
- Vincent Perreten, Title: Animals and dissemination of clinically important antimicrobial resistance: tracking carbapenemases, Swiss Society for Microbiology Annual Congress 2022, SwissTech Convention Center, EPFL, Quartier Nord, Ecublens-Lausanne, August 30 – September 1, 2022, Invited speaker.
- Peter Kuhnert, Title: Characterization and diagnostics of *Wielerella bovis* gen. nov., sp. nov. associated with bovine endocarditis, Tagung der DVG-Fachgruppe AVID Schwerpunkt Bakteriologie, Kloster Banz, September 14-16, 2022, Invited Talk.
- Gudrun Overesch, Title: Antimicrobial resistance – Current Trends and European Activities, 6th Congress of the European Association of Veterinary Laboratory Diagnosticians, October 24-26, 2022, Sevilla, Spain, Invited Key Note speaker.
- Gudrun Overesch, Title: New Technologies in Veterinary Microbiology Diagnostics. Online course (https://kedivimaua.gr/programs/new-technologies-in-veterinary-laboratory-diagnostics/) endorsed by the European College of Veterinary Microbiology (ECVM)
- Gudrun Overesch, Title: MRSA, 9. Schweizerische Tierärztetage 2022, Basel, Schweiz
- Sonja Kittl, Title: EP und APP beim Schwein: Labordiagnostik., 9. Schweizerische Tierärztetage 2022, Basel, Schweiz
- Sonja Kittl, Title: Salmonellose bei Rind und Schwein: Labordiagnostik. 9. Schweizerische Tierärztetage 2022, Basel, Schweiz
- Sonja Kittl, Title: Coxiellose Labordiagnostik. 9. Schweizerische Tierärztetage 2022, Basel, Schweiz

7.3 Outreach activities

- Fabien Labroussaa participated in the Night of Research in presenting the Multidisciplinary Center for Infectious Diseases (MCID), 10th September 2022
- Sergi Torres-Puig, Fabien Labroussaa and Jörg Jores contributed two *Mycoplasma* chapters for: A child-centric microbiology education framework
- Jörg Jores, Title: Bernese pioneering work in cloning of the Coronavirus: the potential of synthetic genomics, Lecture series: University for Seniors, University of Bern-Audimax, 16th December 2022

7.4 Competitive Grants Awarded

- Donor: Multidisciplinary Center for Infectious Diseases (MCID); Project title: 'MCID Core Activity BioPreparedness BioBank? Budget allocation: 665,688.75 CHF; Duration: 6/2022-5/2025; Principal Investigator: Steven Leib, Co-Principal Investigator: Joerg Jores; IVB staff: Joerg Jores, Paraskevi Pramateftaki, Sergi Torres-Puig
- Donor: Multidisciplinary Center for Infectious Diseases (MCID); Project title: 'Use of tailor-made bacteriophages for the treatment of infections caused by multi-drug resistant bacteria, Budget allocation: 300,000 CHF; Duration: 3/2022-2/2025; Principal Investigator: Fabien Labroussaa, Co-Principal Investigator: Stephen Leib; IVB staff: Fabien Labroussaa, Jérémy Cherbuin

7.5 Other funding

- Donor: Federal Food Safety and Veterinary Office (Switzerland); Project title: "Wirksamkeit von IntraCare-Produkten gegen die Moderhinke der Schafe in der Schweiz", Budget: 134,462 CHF; Duration: 2/2022-7/2023; Principal investigator: Peter Kuhnert; IVB staff: Nadia Loosli, Isabelle Brodard

8 Organization Chart (Organigram)