UNIVERSITÄT BERN

# ANNUAL REPORT 2019

# Institute of Veterinary Bacteriology


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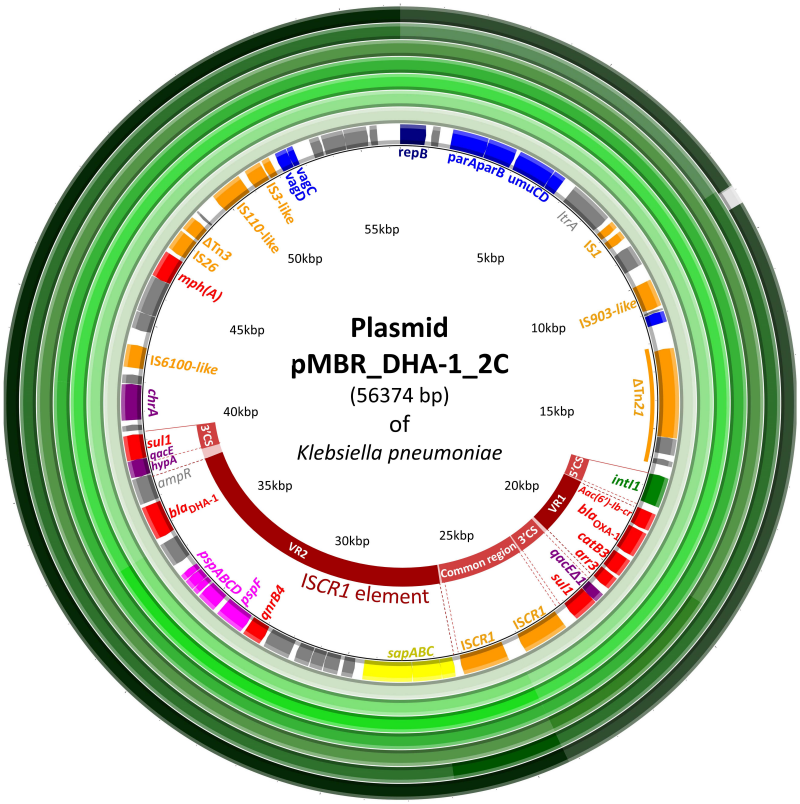

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# 1 Preface

The Institute of Veterinary Bacteriology is delighted to distribute its Annual Report summarizing the activities and publications of 2019. It features the achievements of our staff, students and collaborators.

The year 2019 was devoted to update our biosafety measures. Therefore, we allocated institutional resources to decontaminate our BSL-3 laboratory for subsequent refurbishment. Moreover we updated our biosafety-related documentation with respect to risk assessments. Three people attended biosafety level 2 and 3 courses to increase the knowledge base of biosafety at the institute. We employed an bioinformatician (Hatice Akarsu Egger) and a lab manager (Julia Bolliger) to adapt to a changing scientific landscape and to streamline lab tasks, respectively.

Regarding our teaching commitments we taught students of the VETSUISSE and the SCIENCE faculty at the University of Bern. Besides the undergraduate education, we also contributed to postgraduate education, which is reflected by 3 master degree, 5 DVM degrees and 1 PhD degree. Additionally, we introduced the written exam in bacteriology, which was appreciated by the veterinary students.

Finally, we contributed as every year to the provision of 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, its partners, and customers who made 2019 again a successful year for veterinary bacteriology in Bern.

Bern 17th June 2020 Jörg Jores

# 2 Research Units

## 2.1 Host-Pathogen Interactions

### 2.1.1 Akarsu, H., Aguilar-Bultet, L., Falquet, L., deltaRpkm: an R package for a rapid detection of differential gene presence between related bacterial genomes. BMC Bioinformatics 20, 621 (2019).

*Collaborators:* Department of Biology, University of Fribourg, Fribourg, Switzerland

*Abstract:* Background: Comparative genomics has seen the development of many software performing the clustering, polymorphism and gene content analysis of genomes at different phylogenetic levels (isolates, species). These tools rely on de novo assembly and/or multiple alignments that can be computationally intensive for large datasets. With a large number of similar genomes in particular, e.g., in surveillance and outbreak detection, assembling each genome can become a redundant and expensive step in the identification of genes potentially involved in a given clinical feature.

Results: We have developed deltaRpkm, an R package that performs a rapid differential gene presence evaluation between two large groups of closely related genomes. Starting from a standard gene count table, deltaRpkm computes the RPKM per gene per sample, then the inter-group *δRPKM* values, the corresponding median *δRPKM* (m) for each gene and the global standard deviation value of m ($s_m$). Genes with $m > = 2 * s_m$ (standard deviation s of all the m values) are considered as "differentially present" in the reference genome group. Our simple yet effective method of differential RPKM has been successfully applied in a recent study published by our group (N = 225 genomes of *Listeria monocytogenes*) (Aguilar-Bultet et al. Front Cell Infect Microbiol 8:20, 2018).

Conclusions: To our knowledge, deltaRpkm is the first tool to propose a straightforward inter-group differential gene presence analysis with large datasets of related genomes, including non-coding genes, and to output directly a list of genes potentially involved in a phenotype.

### 2.1.2 Alinaitwe, L., Kankya, C., Allan, K. J., Rodriguez-Campos, S., Torgerson, P., Dreyfus, A., Bovine leptospirosis in abattoirs in Uganda: Molecular detection and risk of exposure among workers. Zoonoses Public Health 66, 636-646 (2019).

*Collaborators:* College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, Kampala, Uganda; Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK; Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland

*Abstract:* Leptospirosis is a zoonotic bacterial disease reported worldwide. In Uganda, seropositivity has been reported in both humans and domesticated animals, including cattle.

However, it remains unknown whether cattle are shedding leptospires and thus acting as potential source for human leptospirosis. We conducted this cross-sectional study in two cattle abattoirs in Kampala, Uganda between June and July 2017. Kidney and urine samples from 500 cattle sourced from across the country were analysed by real-time PCR to establish the prevalence of Leptospira-positive cattle and risk of exposure to abattoir workers. The species of infecting *Leptospira* was determined by amplification of *secY* gene and compared to reference sequences published in GenBank. Of 500 cattle tested, 36 (7.2%) had *Leptospira* DNA in their kidneys (carriers), 29 (5.8%) in their urine (shedders); with an overall prevalence (kidney and/or urine) of 8.8%. *Leptospira borgpetersenii* was confirmed as the infecting species in three cattle and *Leptospira kirschneri* in one animal. Male versus female cattle (OR = 3, p-value 0.003), exotic versus local breeds (OR = 21.3, p-value 0.002) or cattle from Western Uganda (OR = 4.4, p-value 0.001) and from regions across the border (OR = 3.3, p-value 0.032) versus from the central region were more likely to be *Leptospira*-positive. The daily risk of exposure of abattoir workers to ≥1 (kidney and/or urine) positive carcass ranged from 27% (95% credibility interval 18.6-52.3) to 100% (95% CI 91.0-100.0), with halal butchers and pluck inspectors being at highest risk. In conclusion, cattle slaughtered at abattoirs in Uganda carry and shed pathogenic *Leptospira* species; and this may pose occupation-related risk of exposure among workers in these abattoirs, with workers who handle larger numbers of animals being at higher risk.

### 2.1.3 Alsaaod, M., Locher, I., Jores, J., Grimm, P., Brodard, I., Steiner, A., Kuhnert, P., Detection of specific *Treponema* species and *Dichelobacter nodosus* from digital dermatitis (Mortellaro's disease) lesions in Swiss cattle. Schweiz Arch Tierheilkd 161, 207-215 (2019).

*Collaborators:* Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland; Swiss Bovine Health Service (RGD), Switzerland.

*Abstract:* The aim of this study was to determine the prevalence of the three *Treponema* species as well as *D. nodosus* in Digital dermatitis (DD) and slurry of Swiss cattle using PCR. A total of 86 specimens from 24 farms were enrolled in the study. Slurry samples from 21 DD-affected and one unaffected farm were collected to assess the potential of environmental transmission. Nested and real-time PCR were performed from the specimens to detect *Treponema* species and *D. nodosus*, respectively. The DD-stages were positive for at least one or more of the DD-associated *Treponema* species in 50 of 61 cases (82.0%) and in 9 of 25 cases (36.0%) in unaffected animals. Infected animals with small focal active lesions showed a significantly lower prevalence (14.8%) compared to the other DD stages (67.2%; P=0.011). Most prevalent was *T. phagedenis* (65.1%). *D. nodosus* was detected in 51.8% of clinical DD lesions and 24.1% in unaffected cases, but its presence was not significantly associated with the various DD-stages. All samples positive for *D. nodosus* contained the acid protease gene *aprB2* but were

negative for *aprV2*, the latter associated with virulence in sheep foot rot. Control farms were negative for all DD-associated *Treponema* species while positive for *aprB2* and negative for *aprV2*. The presence of *aprB2* suggests it is ubiquitous in the animal environment. With respect to the slurry samples, three out of 21 specimens (14.3%) were positive for one or more of the DD-associated *Treponema* species and eleven out of 21 specimens (52.4%) were positive for *aprB2* and negative for *aprV2* of *D. nodosus*. In conclusion, an association was found between the presence of clinical DD and specific *Treponema* species, while for *D. nodosus* no such link with DD lesions could be observed.

### 2.1.4 Baby, V., Labroussaa, F., Lartigue, C., Rodrigue, S., Synthetic chromosomes: rewriting the code of life. Med Sci (Paris) 35, 753-760 (2019).

*Collaborators:* INRA, UMR 1332 de biologie du fruit et pathologie, 71 avenue E. Bourlaux, 33140 Villenave d'Ornon, France - Univ. Bordeaux, UMR 1332 de biologie du fruit et pathologie, 71 avenue E. Bourlaux 33140 Villenave d'Ornon, France; Departement de biologie, Universite de Sherbrooke, 2500 boulevard de l'Universite, J1K 2R1 Sherbrooke, Quebec, Canada.

*Abstract:* The past decade has seen vast improvements in DNA synthesis and assembly methods. The creation of synthetic DNA molecules is becoming easier and more affordable, such that entire chromosomes can now be synthesized. These advances mark the beginning of synthetic genomics, a new discipline interested in the construction of complete genomes tailored for the study and application of biological systems. From viral genome synthesis to the reconstruction of the yeast 16 chromosomes, we discuss the main discoveries, the regulations and ethical considerations along with the potential of this emerging discipline for the future.

### 2.1.5 Balestrin, E., Kuhnert, P., Wolf, J. M., Wolf, L. M., Fonseca, A. S. K., Ikuta, N., Lunge, V. R., Siqueira, F. M., Clonality of *Mycoplasma hyopneumoniae* in swine farms from Brazil. Vet Microbiol 238, 108434 (2019).

*Collaborators:* Laboratory of Molecular Diagnostic, Lutheran & Laboratory of Molecular Diagnostic, Lutheran University from Brazil (ULBRA), Canoas, Rio Grande do Sul, Brazil; Simbios Biotecnologia, Cachoeirinha, Rio Grande do Sul, Brazil; Laboratory of Veterinary Bacteriology, Veterinary Pathology Department, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

*Abstract: Mycoplasma hyopneumoniae* causes enzootic pneumonia (EP) in swine, a disease related to high economic losses in production systems. Epidemiological spread of *M. hyopneumoniae* clones was studied by multi-locus sequence typing (MLST) in several swine production regions but so far not in South America. Using MLST, we have therefore investigated *M. hyopneumoniae* clones circulating in farms from three main swine production

regions in Brazil. Porcine lungs samples were collected between 2015 and 2016 in farms with EP outbreaks. Three geographically distant regions were selected, and 67 *M. hyopneumoniae* positive samples, each one from a different farm, were included in the study. The occurrence of five sequence types (ST) was demonstrated and the majority of the samples were identified as ST-69 (n=60; 89.5%), followed by ST-70 (n=3; 4.5%), ST-123 (n=2; 3%), ST-124 (n=1; 1.5%) and ST-127 (n=1; 1.5%). There was no association of any specific ST with region or production system. The five STs were all new ones, probably representing unique Brazilian clones. ST-69 and ST-70 on one side and ST-123 and ST-124 on the other side are phylogenetically close, while ST-127 is singleton. In conclusion, our results showed a low variability and high clonality of *M. hyopneumoniae* genotypes from Brazilian farms affected by EP.

### 2.1.6 Baschera, M., Cernela, N., Stevens, M. J. A., Liljander, A., Jores, J., Corman, V. M., Nuesch-Inderbinen, M., Stephan, R., Shiga toxin-producing *Escherichia coli* (STEC) isolated from fecal samples of African dromedary camels. One Health 7, 100087 (2019).

*Collaborators:* Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Switzerland; International Livestock Research Institute, PO Box 30709, 00100 Nairobi, Kenya; Charite-Universitatsmedizin Berlin, Humboldt-Universitat zu Berlin, Berlin Institute of Health, Institute of Virology, Berlin, Germany

*Abstract:* Shiga toxin-producing *Escherichia coli* (STEC) cause gastrointestinal illnesses including non-bloody or bloody diarrhoea, haemorrhagic colitis (HC), and the haemolytic uremic syndrome (HUS). To investigate the occurrence of STEC among grazing dromedaries from Kenya, *E. coli* isolated from fecal matter collected from 163 dromedaries on a large ranch were screened for the presence of *stx1* and *stx2*. STEC strains were isolated and serotyped. Isolates were subjected to PCR for the subtyping of *stx* genes and for the detection of *eae* and *ehx*. In addition, whole genome sequencing (WGS) was carried out to detect further virulence genes and to determine the multilocus sequence types (MLST). Antimicrobial resistance profiles were determined by disk diffusion. STEC was isolated from 20 (12.3%) of the fecal samples. Thereof, nine (45%) isolates were STEC O156:H25, three (15%) isolates typed STEC O43:H2. The remaining isolates occurred as single serotypes or were O non-typeable. Eleven (55%) of the isolates harboured *stx2a*, nine (45%) *eae*, and 14 (70%) *ehx*, respectively. WGS revealed the presence of *iss* in 16 (80%), subAB in four (20%) and astA in two (10%) of the isolates, Furthermore, *espA, tccP, nleA, nleB, tccP*, and *tir* were found exclusively among STEC O156:H25. Eleven different sequence types (ST) were detected. The most prominent was ST300/ST5343, which comprised STEC O156:H25. All STEC isolates were pan susceptible to a panel of 16 antimicrobial agents. Overall, the results indicate that dromedary camels in Kenya may be reservoirs of STEC, including serotypes possessing virulence markers associated to

disease in humans, such as STEC O156:H25. STEC in camels may represent a health hazard for humans with close contact to camels or to consumers of camel derived foodstuffs, such as unpasteurised camel milk.

### 2.1.7 Jores, J., Ma, L., Ssajjakambwe, P., Schieck, E., Liljander, A., Chandran, S., Stoffel, M. H., Cippa, V., Arfi, Y., Assad-Garcia, N., Falquet, L., Sirand-Pugnet, P., Blanchard, A., Lartigue, C., Posthaus, H., Labroussaa, F., Vashee, S., Removal of a Subset of Non-essential Genes Fully Attenuates a Highly Virulent Mycoplasma Strain. Front Microbiol 10, 664 (2019).

*Collaborators:* International Livestock Research Institute, Nairobi, Kenya; J. Craig Venter Institute, Rockville, MD, USA; College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda; Division of Veterinary Anatomy, University of Bern, Bern, Switzerland; UMR 1332 - Biologie du Fruit et Pathologie, Institut National de la Recherche Agronomique, Villenave-d'Ornon, France; UMR 1332 - Biologie du Fruit et Pathologie, Universite de Bordeaux, Villenave-d'Ornon, France; Biochemistry Unit, Swiss Institute of Bioinformatics, University of Fribourg, Fribourg, Switzerland; Institute of Animal Pathology (COMPATH), University of Bern, Bern, Switzerland.

*Abstract:* Mycoplasmas are the smallest free-living organisms and cause a number of economically important diseases affecting humans, animals, insects, and plants. Here, we demonstrate that highly virulent *Mycoplasma mycoides* subspecies *capri* (*Mmc*) can be fully attenuated via targeted deletion of non-essential genes encoding, among others, potential virulence traits. Five genomic regions, representing approximately 10% of the original *Mmc* genome, were successively deleted using Saccharomyces cerevisiae as an engineering platform. Specifically, a total of 68 genes out of the 432 genes verified to be individually non-essential in the JCVI-Syn3.0 minimal cell, were excised from the genome. *In vitro* characterization showed that this mutant was similar to its parental strain in terms of its doubling time, even though 10% of the genome content were removed. A novel *in vivo* challenge model in goats revealed that the wild-type parental strain caused marked necrotizing inflammation at the site of inoculation, septicemia and all animals reached endpoint criteria within 6 days after experimental infection. This is in contrast to the mutant strain, which caused no clinical signs nor pathomorphological lesions. These results highlight, for the first time, the rational design, construction and complete attenuation of a *Mycoplasma* strain via synthetic genomics tools. Trait addition using the yeast-based genome engineering platform and subsequent *in vitro* or *in vivo* trials employing the *Mycoplasma* chassis will allow us to dissect the role of individual candidate *Mycoplasma* virulence factors and lead the way for the development of an attenuated designer vaccine.

### 2.1.8 Jores, J., Schieck, E., Liljander, A., Sacchini, F., Posthaus, H., Lartigue, C., Blanchard, A., Labroussaa, F., Vashee, S., *In vivo* role of capsular polysaccharide in *Mycoplasma mycoides*. J Infect Dis 219, 1559-1563 (2019).

*Collaborators:* International Livestock Research Institute, Nairobi, Kenya; Institute of Veterinary Pathology, University of Bern, Switzerland; French National Institute for Agricultural Research, Villenave d'Ornon, France; University of Bordeaux, UMR 1332 de Biologie du Fruit et Pathologie, Villenave d'Ornon, France; J. Craig Venter Institute, Rockville, USA

*Abstract:* Capsular polysaccharides have been confirmed to be an important virulence trait in many gram-positive and gram-negative bacteria. Similarly, they are proposed to be virulence traits in minimal *Mycoplasma* that cause disease in humans and animals. In the current study, goats were infected with the caprine pathogen *Mycoplasma mycoides* subsp. *capri* or an engineered mutant lacking the capsular polysaccharide, galactofuranose. Goats infected with the mutant strain showed only transient fever. In contrast, 5 of 8 goats infected with the parental strain reached end-point criteria after infection. These findings confirm that galactofuranose is a virulence factor in *M. mycoides*.

### 2.1.9 Josi, C., Burki, S., Vidal, S., Dordet-Frisoni, E., Citti, C., Falquet, L., Pilo, P., Large-Scale Analysis of the *Mycoplasma bovis* Genome Identified Non-essential, Adhesion- and Virulence-Related Genes. Front Microbiol 10, 2085 (2019).

*Collaborators:* UMR 1225, IHAP, Universite de Toulouse, INRA, ENVT, Toulouse, France; Faculty of Science and Medicine, Swiss Institute of Bioinformatics, University of Fribourg, Fribourg, Switzerland.

*Abstract: Mycoplasma bovis* is an important pathogen of cattle causing bovine mycoplasmosis. Clinical manifestations are numerous, but pneumonia, mastitis, and arthritis cases are mainly reported. Currently, no efficient vaccine is available and antibiotic treatments are not always satisfactory. The design of new, efficient prophylactic and therapeutic approaches requires a better understanding of the molecular mechanisms responsible for *M. bovis* pathogenicity. Random transposon mutagenesis has been widely used in *Mycoplasma* species to identify potential gene functions. Such an approach can also be used to screen genomes and search for essential and non-essential genes for growth. Here, we generated a random transposon mutant library of *M. bovis* strain JF4278 containing approximately 4000 independent insertion sites. We then coupled high-throughput screening of this mutant library to transposon sequencing and bioinformatic analysis to identify *M. bovis* non-essential, adhesion- and virulence-related genes. Three hundred and fifty-two genes of *M. bovis* were assigned as essential for growth in rich medium. Among the remaining non-essential genes, putative virulence-related factors were subsequently identified. The complete mutant library was screened for adhesion using primary bovine mammary gland epithelial cells. Data from this assay resulted in a list of conditional-

essential genes with putative adhesion-related functions by identifying non-essential genes for growth that are essential for host cell-adhesion. By individually assessing the adhesion capacity of six selected mutants, two previously unknown factors and the adhesin TrmFO were associated with a reduced adhesion phenotype. Overall, our study (i) uncovers new, putative virulence-related genes; (ii) offers a list of putative adhesion-related factors; and (iii) provides valuable information for vaccine design and for exploring *M. bovis* biology, pathogenesis, and host-interaction.

### 2.1.10 Kuhnert, P., Cippa, V., Hardi-Landerer, M. C., Schmicke, M., Ulbrich, S., Locher, I., Steiner, A., Jores, J., Early Infection Dynamics of *Dichelobacter nodosus* During an Ovine Experimental Footrot In Contact Infection. Schweiz Arch Tierheilkd 161, 465-472 (2019).

*Collaborators:* ETH Zurich, Animal Physiology, Institute of Agricultural Sciences, Zurich, Switzerland; Clinic for Cattle, University of Veterinary Medicine Hannover, Germany; Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Switzerland

*Abstract:* INTRODUCTION: Ovine footrot caused by *Dichelobacter nodosus* is a highly contagious and painful disease representing an economic as well as an animal welfare problem. In order to get more information on the infection dynamics, 26 lambs and 4 ewes enrolled in an in-contact infection trial were monitored over two weeks for the presence of *D. nodosus*-specific DNA. Two *D. nodosus*-positive ewes were housed together with 13 confirmed negative lambs. The control group consisted of another 13 lamb siblings and two confirmed *D. nodosus*-negative ewes. Every foot of all sheep was sampled seven times over the two weeks experiment period and subsequently analyzed for the presence of *D. nodosus* by quantitative real-time PCR. The control group was negative at the beginning and the end of the experiment and showed no clinical symptoms of footrot. The two positive ewes showed a high, but hundred fold differing level of virulent *D. nodosus* that remained constant over time with one of the ewes being also weakly positive for benign *D. nodosus*. All lambs of the infection group were positive for virulent *D. nodosus* at 14 days post infection (dpi). The first positive animals were observed on 3 dpi. The *D. nodosus* load remained at a low level and only increased in a few lambs at the end of the trial. Five of the contact lambs showed suspicious clincal signs (score 1-2) at 14 dpi corroborating the PCR results and indicating that the disease starts as early as two weeks after contact with positive sheep.

### 2.1.11 Labroussaa, F., Baby, V., Rodrigue, S., Lartigue, C., Whole genome transplantation: bringing natural or synthetic bacterial genomes back to life. Med

**Sci (Paris) 35, 761-770 (2019).**

*Collaborators:* INRA, UMR 1332 de biologie du fruit et pathologie, 71 avenue E. Bourlaux, 33140 Villenave d'Ornon, France - Univ. Bordeaux, UMR 1332 de biologie du fruit et pathologie, 71 avenue E. Bourlaux 33140 Villenave d'Ornon, France; Departement de biologie, Universite de Sherbrooke, 2500 boulevard de l'Universite, J1K 2R1 Sherbrooke, Quebec, Canada.

*Abstract:* The development of synthetic genomics (SG) allowed the emergence of several groundbreaking techniques including the synthesis, assembly and engineering of whole bacterial genomes. The successful implantation of those methods, which culminated in the creation of JCVI-syn3.0 the first nearly minimal bacterium with a synthetic genome, mainly results from the use of the yeast *Saccharomyces cerevisiae* as a transient host for bacterial genome replication and modification. Another method played a key role in the resounding success of this project: bacterial genome transplantation (GT). GT consists in the transfer of bacterial genomes cloned in yeast, back into a cellular environment suitable for the expression of their genetic content. While successful using many mycoplasma species, a complete understanding of the factors governing GT will most certainly help unleash the power of the entire SG pipeline to other genetically intractable bacteria.

**2.1.12 Lartigue, C., Valverde Timana, Y., Labroussaa, F., Schieck, E., Liljander, A., Sacchini, F., Posthaus, H., Batailler, B., Sirand-Pugnet, P., Vashee, S., Jores, J.*, Blanchard, A.*, Attenuation of a Pathogenic *Mycoplasma* Strain by Modification of the *obg* Gene by Using Synthetic Biology Approaches. mSphere 4 (2019). *equal contribution**

*Collaborators:* UMR 1332 de Biologie du Fruit et Pathologie, Villenave d'Ornon, France; INRA, UMR 1332 de Biologie du Fruit et Pathologie, Villenave d'Ornon, France; International Livestock Research Institute, Nairobi, Kenya; Institute of Veterinary Pathology, University of Bern, Bern, Switzerland; J. Craig Venter Institute, Rockville, Maryland, USA.

*Abstract: Mycoplasma* species are responsible for several economically significant livestock diseases for which there is a need for new and improved vaccines. Most of the existing mycoplasma vaccines are attenuated strains that have been empirically obtained by serial passages or by chemical mutagenesis. The recent development of synthetic biology approaches has opened the way for the engineering of live mycoplasma vaccines. Using these tools, the essential GTPase-encoding gene *obg* was modified directly on the *Mycoplasma mycoides* subsp. *capri* genome cloned in yeast, reproducing mutations suspected to induce a temperature-sensitive (TS(+)) phenotype. After transplantation of modified genomes into a recipient cell, the phenotype of the resulting *M. mycoides* subsp. *capri* mutants was characterized. Single-point obg mutations did not result in a strong TS(+) phenotype in *M. mycoides* subsp. *capri*, but a clone presenting three *obg* mutations was shown to grow with difficulty at temperatures of

>/=40 degrees C. This particular mutant was then tested in a caprine septicemia model of *M. mycoides* subsp. *capri* infection. Five out of eight goats infected with the parental strain had to be euthanized, in contrast to one out of eight goats infected with the obg mutant, demonstrating an attenuation of virulence in the mutant. Moreover, the strain isolated from the euthanized animal in the group infected with the obg mutant was shown to carry a reversion in the obg gene associated with the loss of the TS(+) phenotype. This study demonstrates the feasibility of building attenuated strains of mycoplasma that could contribute to the design of novel vaccines with improved safety.

**2.1.13 Liljander, A., Sacchini, F., Stoffel, M. H., Schieck, E., Stokar-Regenscheit, N., Labroussaa, F., Heller, M., Salt, J., Frey, J., Falquet, L., Goovaerts, D., Jores, J., Reproduction of contagious caprine pleuropneumonia reveals the ability of convalescent sera to reduce hydrogen peroxide production in vitro. Vet Res 50, 10 (2019).**

*Collaborators:* International Livestock Research Institute, Box 30709, Nairobi, 00100, Kenya; Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", via Campo Boario, 64100, Teramo, Italy; Division of Veterinary Anatomy, Vetsuisse Faculty, University of Bern, Langgass-Str. 120, Postfach, 3001, Bern, Switzerland; Institute of Animal Pathology (COMPATH), Vetsuisse Faculty, University of Bern, Langgass-Str. 122, Postfach, 3001, Bern, Switzerland; Friedrich-Loeffler-Institute-Federal Research Institute for Animal Health, Naumburger Str. 96a, 07743, Jena, Germany; GALVmed, Doherty Building, Pentlands Science Park, Bush Loan, Penicuik, Edinburgh, EH26 0PZ, Scotland, UK; Division of Biochemistry, Department of Biology, University of Fribourg and Swiss Institute of Bioinformatics, Chemin du Musee 18, 1700, Fribourg, Switzerland

*Abstract:* Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae* is a severe disease widespread in Africa and Asia. Limited knowledge is available on the pathogenesis of this organism, mainly due to the lack of a robust *in vivo* challenge model and the means to do site-directed mutagenesis. This work describes the establishment of a novel caprine challenge model for CCPP that resulted in 100% morbidity using a combination of repeated intranasal spray infection followed by a single transtracheal infection employing the recent Kenyan outbreak strain ILRI181. Diseased animals displayed CCPP-related pathology and the bacteria could subsequently be isolated from pleural exudates and lung tissues in concentrations of up to $10^9$ bacteria per mL as well as in the trachea using immunohistochemistry. Reannotation of the genome sequence of ILRI181 and $F38^T$ revealed the existence of genes encoding the complete glycerol uptake and metabolic pathways involved in hydrogen peroxide ($H_2O_2$) production in the phylogenetically related pathogen *M. mycoides* subsp. *mycoides*. Furthermore, the expression of L-alpha-glycerophosphate oxidase (GlpO) in vivo was confirmed. In addition, the function of the glycerol metabolism was verified by

measurement of production of $H_2O_2$ in medium containing physiological serum concentrations of glycerol. Peroxide production could be inhibited with serum from convalescent animals. These results will pave the way for a better understanding of host-pathogen interactions during CCPP and subsequent vaccine development.

### 2.1.14 Mehinagic, K., Pilo, P., Vidondo, B., Stokar-Regenscheit, N., Coinfection of Swiss cattle with bovine parainfluenza virus 3 and *Mycoplasma bovis* at acute and chronic stages of bovine respiratory disease complex. J Vet Diagn Invest 31, 674-680 (2019).

*Collaborators:* Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Veterinary Public Health Institute (Vidondo), Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland.

*Abstract:* Viral agents such as bovine respiratory syncytial virus (BRSV) and bovine parainfluenza virus 3 (BPIV-3) are considered primary infectious agents in bovine respiratory disease complex (BRDC). Information regarding the pathogenesis of BRDC is scarce, especially at an advanced chronicity stage, in addition to ongoing coinfection with other primary agents such as *Mycoplasma bovis*. Based on a retrospective review of histology slides from 104 autopsy cases, we classified cases according to type of pneumonia and chronicity. We performed immunohistochemistry (IHC) for BRSV, BPIV-3, and *M. bovis* as well as real-time PCR (rtPCR) for *M. bovis* on lung tissue of all 104 cases and correlated results with the morphologic type of pneumonia. Histomorphologically, 79 cases were classified as bronchopneumonia, 16 as bronchointerstitial pneumonia, and 9 as interstitial pneumonia. In 89 cases, at least 1 of the investigated agents was detected by IHC; 44 of these cases had a coinfection. BPIV-3 was the predominant agent present, as a single infection in 39 cases, and in coinfection with *M. bovis* in 39 cases. Comparing the detection methods for *M. bovis*, rtPCR was more specific and sensitive than IHC. The combination of both methods provided a good visual tool for assessing severity and distribution of *M. bovis* antigen within the tissue. Unlike BRSV, BPIV-3 and *M. bovis* persisted in chronic BRDC, suggesting ongoing impairment of defense mechanisms in the lung.

### 2.1.15 Nicholson, P., Furrer, J., Hassig, M., Strauss, C., Heller, M., Braga-Lagache, S., Frey, J., Production of neutralizing antibodies against the secreted *Clostridium chauvoei* toxin A (CctA) upon blackleg vaccination. Anaerobe 56, 78-87 (2019).

*Collaborators:* Department for Small Animals, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland; Department for BioMedical Research, Faculty of Medicine, University of Bern, Bern, Switzerland

*Abstract: Clostridium chauvoei* is the etiologic agent of blackleg in cattle, inducing fever, severe myonecrosis, oedemic lesions and ultimately death of infected animals. The pathogen often results in such rapid death that antibiotic therapy is futile and thus vaccination is the only efficient strategy in order to control the disease. The beta-barrel pore forming leucocidin *Clostridium chauvoei* toxin A (CctA) is one of the best characterised toxins of *C. chauvoei* and has been shown to be an important virulence factor. It has been reported to induce protective immunity and is conserved across *C. chauvoei* strains collected from diverse geographical locations for more than 50 years. The aim of this study was to identify the location of the CctA toxin during liquid culture fermentation and to use CctA to develop an in vitro assay to replace the current guinea pig challenge assay for vaccine potency in standard batch release procedures. We report that CctA is fully secreted in *C. chauvoei* culture and show that it is found abundantly in the supernatant of liquid cultures. Sera from cattle vaccinated with a commercial blackleg vaccine revealed strong haemolysin-neutralizing activity against recombinant CctA which reached titres of 1000 times 28 days post-vaccination. Similarly, guinea pig sera from an official potency control test reached titres of 600 times 14 days post-vaccination. In contrast, ELISA was not able to specifically measure anti-CctA antibodies in cattle serum due to strong cross-reactions with antibodies against other proteins present pre-vaccination. We conclude that haemolysin-neutralizing antibodies are a valuable measurement for protective immunity against blackleg and have the potential to be a suitable replacement of the guinea pig challenge potency test, which would forego the unnecessary challenge of laboratory animals.

### 2.1.16 Salt, J., Jores, J., Labroussaa, F., Wako, D. D., Kairu-Wanyoike, S. W., Nene, V., Stuke, K., Mulongo, M., Sirand-Pugnet, P., Vaccination against CCPP in East Africa. Vet Rec 185, 272 (2019).

*Collaborators:* GALVmed, Doherty Building, Pentlands Science Park, Bush Loan, Penicuik Edinburgh EH26 0PZ, Scotland; Sidai Africa Limited, PO Box 27256, Nairobi 00100, Kenya; State Department of Livestock, PO Box 34188, Nairobi 00100, Kenya; International Livestock Research Institute, PO Box 30709, Nairobi 00100, Kenya; Internations Development Research Centre (IDRC), 150 Kent Street, PO Box 8500, Ottawa K1G 3H9, Canada; INRA, Universite de Bordeaux, UMR 1332 de Biologie du Fruit et Pathologie, Villenave d'Ornon 33140, France

### 2.1.17 Schumacher, M., Nicholson, P., Stoffel, M. H., Chandran, S., D'Mello, A., Ma, L., Vashee, S., Jores, J., Labroussaa, F., Evidence for the Cytoplasmic Localization of the L-alpha-Glycerophosphate Oxidase in Members of the "*Mycoplasma mycoides*

**Cluster". Front Microbiol 10, 1344 (2019).**

*Collaborators:* Division of Veterinary Anatomy, University of Bern, Bern, Switzerland; J. Craig Venter Institute, Rockville, MD, USA; Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA

*Abstract:* Members of the "*Mycoplasma mycoides* cluster" are important animal pathogens causing diseases including contagious bovine pleuropneumonia and contagious caprine pleuropneumonia, which are of utmost importance in Africa or Asia. Even if all existing vaccines have shortcomings, vaccination of herds is still considered the best way to fight mycoplasma diseases, especially with the recent and dramatic increase of antimicrobial resistance observed in many mycoplasma species. A new generation of vaccines will benefit from a better understanding of the pathogenesis of mycoplasmas, which is very patchy up to now. In particular, surface-exposed virulence traits are likely to induce a protective immune response when formulated in a vaccine. The candidate virulence factor L-alpha-glycerophosphate oxidase (GlpO), shared by many mycoplasmas including *Mycoplasma pneumoniae*, was suggested to be a surface-exposed enzyme in *Mycoplasma mycoides* subsp. *mycoides* responsible for the production of hydrogen peroxide directly into the host cells. We produced a *glpO* isogenic mutant GM12::YCpMmyc1.1-*ΔglpO* using in-yeast synthetic genomics tools including the tandem-repeat endonuclease cleavage (TREC) technique followed by the back-transplantation of the engineered genome into a mycoplasma recipient cell. GlpO localization in the mutant and its parental strain was assessed using scanning electron microscopy (SEM). We obtained conflicting results and this led us to reevaluate the localization of GlpO using a combination of in silico and *in vitro* techniques, such as Triton X-114 fractionation or tryptic shaving followed by immunoblotting. Our *in vitro* results unambiguously support the finding that GlpO is a cytoplasmic protein throughout the "*Mycoplasma mycoides* cluster." Thus, the use of GlpO as a candidate vaccine antigen is unlikely to induce a protective immune response.

**2.1.18 Trüeb, B. S., Gerber, S., Maes, D., Gharib, W. H., Kuhnert, P., Tn-sequencing of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* mutant libraries reveals non-essential genes of porcine mycoplasmas differing in pathogenicity. Vet Res 50, 55 (2019).**

*Collaborators:* Unit Porcine Health Management, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium; Interfaculty Bioinformatics Unit and Swiss, Institute of Bioinformatics, University of Bern, Bern, Switzerland

*Abstract: Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* are two phylogenetically related species colonizing the respiratory tract of pigs but differing in pathogenicity, the basis

of which is not well resolved. We hypothesize that genes belonging to the species-specific portion of the genome and being non-essential during ideal laboratory growth conditions encode possible virulent determinants and are the driver of interspecies differences. To investigate this, transposon mutant libraries were generated for both species and a transposon sequencing (Tn-seq) method for mycoplasmas was established to identify non-essential genes. Tn-seq datasets combined with bidirectional Blastp analysis revealed that 101 out of a total 678 coding sequences (CDS) are species-specific and non-essential CDS of *M. hyopneumoniae* strain F7.2C, while 96 out of a total 751 CDS are species-specific and non-essential CDS in the *M. hyorhinis* strain JF5820. Among these species-specific and non-essential CDS were genes involved in metabolic pathways. In particular, the myo-inositol and the sialic acid pathways were found to be non-essential and therefore could be considered important to the specific pathogenicity of *M. hyopneumoniae* and *M. hyorhinis*, respectively. Such pathways could enable the use of an alternative energy source providing an advantage in their specific niche and might be interesting targets to knock out in order to generate attenuated live vaccines.

### 2.1.19 Weldearegay, Y. B., Muller, S., Hanske, J., Schulze, A., Kostka, A., Ruger, N., Hewicker-Trautwein, M., Brehm, R., Valentin-Weigand, P., Kammerer, R., Jores, J., Meens, J., Host-Pathogen Interactions of *Mycoplasma mycoides* in Caprine and Bovine Precision-Cut Lung Slices (PCLS) Models. Pathogens 8 (2019).

*Collaborators:* Institute for Microbiology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, 30173 Hannover, Germany; Institute of Immunology, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, 17493 Greifswald-Insel Riems, Germany; Institute for Pathology, University of Veterinary Medicine Hannover, 30559 Hannover, Germany; Institute of Anatomy, University of Veterinary Medicine Hannover, 30173 Hannover, Germany

*Abstract:* Respiratory infections caused by mycoplasma species in ruminants lead to considerable economic losses. Two important ruminant pathogens are *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*), the aetiological agent of contagious bovine pleuropneumonia and *Mycoplasma mycoides* subsp. *capri* (*Mmc*), which causes pneumonia, mastitis, arthritis, keratitis, and septicemia in goats. We established precision cut lung slices (PCLS) infection model for *Mmm* and *Mmc* to study host-pathogen interactions. We monitored infection over time using immunohistological analysis and electron microscopy. Moreover, infection burden was monitored by plating and quantitative real-time PCR. Results were compared with lungs from experimentally infected goats and cattle. Lungs from healthy goats and cattle were also included as controls. PCLS remained viable for up to two weeks. Both subspecies adhered to ciliated cells. However, the titer of *Mmm* in caprine PCLS decreased over time, indicating species specificity of *Mmm*. *Mmc* showed higher tropism to sub-bronchiolar tissue in caprine PCLS, which increased in a time-dependent manner. Moreover, *Mmc* was abundantly observed

on pulmonary endothelial cells, indicating partially, how it causes systemic disease. Tissue destruction upon prolonged infection of slices was comparable to the *in vivo* samples. Therefore, PCLS represents a novel *ex vivo* model to study host-pathogen interaction in livestock mycoplasma.

## 2.2 Molecular Epidemiology and Infectiology

### 2.2.1 Albert, E., Biksi, I., Nemet, Z., Csuka, E., Kelemen, B., Morvay, F., Bakos, Z., Bodo, G., Toth, B., Collaud, A., Rossano, A., Perreten, V., Outbreaks of a Methicillin-resistant *Staphylococcus aureus* clone ST398-t011 in a Hungarian equine clinic: Emergence of rifampicin and chloramphenicol resistance after treatment with these antibiotics. Microb Drug Resist 25, 1219-1226 (2019).

*Collaborators:* Diagnostic Laboratory, Department and Clinic of Production Animal Medicine, University of Veterinary Medicine Budapest, Üllő, Hungary; Department and Clinic of Equine Medicine, University of Veterinary Medicine Budapest, Üllő, Hungary.

*Abstract:* Between July 2011 and May 2016, a total of 40 *Staphylococcus aureus* strains originating from 36 horses were confirmed as methicillin resistant (methicillin-resistant *Staphylococcus aureus* [MRSA]) in a university equine clinic. An additional 10 MRSA strains from 36 samples of clinic workers were obtained in October 2017. The first equine isolate represented the sequence type ST398, *spa*-type t011, and SCC*mec* IV. This isolate was resistant to a wide spectrum of antimicrobial agents. MRSA strains with the same genotype and with very similar resistance profiles were isolated on 21 more occasions from September 2013 to September 2014. A second outbreak occurred from May 2015 until May 2016. The first isolate in this second outbreak shared the same genotype, but was additionally resistant to chloramphenicol. The second isolate from August 2015 also showed resistance to rifampicin. The clone was isolated 18 times. Most of the human isolates shared the same genotype as the isolates from horses and their resistance patterns showed only slight differences. We can conclude that the MRSA-related cases at the Department and Clinic of Equine Medicine were all nosocomial infections caused by the same clonal lineage belonging to the clonal complex 398. The clonal complex 398 of equine origin is reported for the first time in Hungary. In addition, our observation of the emergence of new resistance to antimicrobial agents within the clonal lineage after treatment with antibiotics is of concern. Strict hygiene regulations have been introduced to lower the incidence of MRSA isolation and the related clinical disease.

### 2.2.2 Brilhante, M., Perreten, V., Donà, V., Multidrug resistance and multivirulence plasmids in enterotoxigenic and hybrid Shiga toxin-producing/enterotoxigenic *Escherichia coli* isolated from diarrheic pigs in Switzerland. Vet J 244, 60-68

**(2019).**

*Abstract:* Enterovirulent Escherichia coli infections cause significant losses in the pig industry. However, information about the structures of the virulence and multidrug resistance (MDR) plasmids harboured by these strains is sparse. In this study, we used whole-genome sequencing with PacBio and Illumina platforms to analyse the molecular features of the multidrug-resistant enterotoxigenic *E. coli* (ETEC) strain 14OD0056 and the multidrug-resistant hybrid Shiga toxin-producing/enterotoxigenic *E. coli* (STEC/ETEC) strain 15OD0495 isolated from diarrheic pigs in Switzerland. Strain 14OD0056 possessed three virulence plasmids similar to others previously found in ETEC strains, while 15OD0495 harboured a 119-kb multivirulence IncFII/IncX1 hybrid STEC/ETEC plasmid (p15ODTXV) that co-carried virulence genes of both ETEC and STEC pathotypes, confirming the key role of plasmids in the emergence of hybrid pathotypes. All resistance genes of 14OD0056 that conferred resistance to ampicillin (*bla*$_{\text{TEM-1b}}$), gentamicin (*aac(3)-IIa*), kanamycin (*aph(3')-Ia*), sulfonamide (*sul1* and *sul2*), streptomycin (*aph(3")-Ib*, *aph(6)-Id*), tetracycline (*tet*(B)) and trimethoprim (*dfrA1*) were identified on a single 207-kb conjugative MDR plasmid of incompatibility group (Inc) IncHI1/IncFIA (p14ODMR). Strain 15OD0495 carried two antimicrobial resistance plasmids (p15ODAR and p15ODMR). The 99-kb IncI1 plasmid p15ODAR harboured only aminoglycoside resistance genes (*aac(3)-IIa*, *aph(3")-Ib*, *aph(6)-Id*, *aph(4)-Ia*), whilst the 49-kb IncN MDR plasmid p15ODMR carried genes conferring resistance to ampicillin (*bla*$_{\text{TEM-1b}}$), sulfonamide (*sul2*), streptomycin (*aph(6)-Id*), tetracycline (*tet*(A)) and trimethoprim (*dfrA14*). Filter mating assays showed that p14ODMR, p15ODMR and p15ODAR were conjugative at room temperature and 37°C. The co-localization of multiple resistance genes on MDR conjugative plasmids such as p14ODMR and p15ODMR poses the risk of simultaneous selection of several resistance traits during empirical treatment. Thus, preventive strategies and targeted therapy following antibiotic susceptibility testing should be encouraged to avoid further dissemination of such plasmids.

### 2.2.3 Brilhante, M., Donà, V., Overesch, G., Endimiani, A., Perreten, V., Characterisation of a porcine *Escherichia coli* strain from Switzerland carrying *mcr-1* on a conjugative multidrug resistance IncHI2 plasmid. J Glob Antimicrob Resist 16, 123-124 (2019)

*Collaborators:* Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern, Switzerland

*Abstract:* In Switzerland, *mcr* genes have so far only been identified in *Escherichia coli* isolates from human, imported food and environmental sources. We characterised the genetic background of the first colistin-resistant porcine strain of *E. coli* carrying *mcr-1* on a conjugative multidrug resistance (MDR) plasmid pRDB9 in Switzerland using WGS. Plasmid pRDB9 carried antimicrobial resistance genes (ARGs), which were associated with resistance

to ampicillin (*bla*$_{\text{TEM-1}}$), chloramphenicol (*cmlA1*), colistin (*mcr-1*), gentamicin [*aac(3)-IIa*], kanamycin [*aph(3')-Ia*], sulfamethoxazole (*sul1*, *sul2*, *sul3*), trimethoprim (*dfrA1*) and tetracycline [*tet*(A)]. Co-localisation of *mcr-1* with additional ARGs on a conjugative plasmid is concerning since all of these ARGs conferred resistance to several antibiotics commonly used in pig husbandry.

### 2.2.4 Chanchaithong, P., Perreten, V., Am-In, N., Lugsomya, K., Tummaruk, P., Prapasarakul, N., Molecular Characterization and Antimicrobial Resistance of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* Isolates from Pigs and Swine Workers in Central Thailand. Microb Drug Resist 25, 1382-1389 (2019).

*Collaborators:* Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; Diagnosis and Monitoring of Animal Pathogen Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

*Abstract:* This study presents molecular characteristics of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) from pigs and swine workers in central Thailand. Sixty-three MRSA isolates were recovered from pigs ($n$ = 60) and humans ($n$ = 3). Two major LA-MRSA lineages, including sequence type (ST) 398 and clonal complex 9 (ST9 and ST4576, a novel single-locus variant of ST9), were identified. ST398 had *spa* type t034 ($n$ = 55). ST9 and ST4576 had t337 ($n$ = 8) and carried staphylococcal cassette chromosome *mec* (SCC*mec*) IX only. MRSA-ST398-t034 contained various SCC*mec*, including SCC*mec* V ($n$ = 42), a novel SCC*mec* composite island ($n$ = 12), and a nontypeable SCC*mec* ($n$ = 1). All isolates were multidrug resistant and carried common resistance genes found in LA-MRSA. This is the first report of the presence of swine MRSA ST398 and multidrug resistance gene *cfr* in MRSA ST9 in Thailand. With identical molecular characteristics, pigs could be a source of MRSA ST398 spread to humans. A minor variation of genetic features and resistance gene carriage in both lineages represented a heterogeneous population and evolution of the endemic clones. A monitoring program and farm management, with prudent antimicrobial uses, should be implemented to reduce spreading. Strict hygiene and personal protection are also necessary to prevent transfer of LA-MRSA to humans.

### 2.2.5 Chanchaithong, P., Perreten, V., Schwendener, S., *Macrococcus canis* contains recombinogenic methicillin resistance elements and the *mecB* plasmid found in

***Staphylococcus aureus*. J Antimicrob Chemother 74, 2531-2536 (2019).**

*Collaborators:* Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand; Diagnosis and Monitoring of Animal Pathogen Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

*Abstract:* The genetic context of *mecB* in two *Macrococcus canis* strains from dogs was analysed and compared with those found in other *Macrococcus* and *Staphylococcus* species. Whole genomes of the *M. canis* strains Epi0076A and KM0218 were sequenced using next-generation sequencing technologies. Multiple PCRs and restriction analysis confirmed structures of *mecB*-containing elements, circularization and recombination of *mecB* subunits. Both *M. canis* strains contained novel composite pseudo (Ψ) staphylococcal cassette chromosome *mec* (SCC*mec*) elements. Integration site sequences for SCC flanked and subdivided composite ΨSCC*mec*$_{Epi0076A}$ (69569 bp) into ΨSCC1$_{Epi0076A}$-ΨSCC*mec*Epi0076A-ΨSCC2$_{Epi0076A}$ and composite ΨSCC*mec*$_{KM0218}$ (24554 bp) into ΨSCC$_{KM0218}$-ΨSCC*mec*$_{KM0218}$. Putative γ-haemolysin genes (*hlgB* and *hlgC*) were found at the 3' end of both composite elements. ΨSCC*mec*KM0218 contained a complete *mecB* gene complex (*mecIm-mecR1m-mecB-blaZm*) downstream of a new IS*21*-family member (IS*Maca1*). ΨSCC*mec*$_{Epi0076A}$ carried a *blaZm*-deleted *mecB* gene complex similar to that reported in *'Macrococcus goetzii'* CCM4927$^{T}$. A second *mecB* gene was found on the 81325 bp MDR plasmid pKM0218 in KM0218. This plasmid contained a complete Tn*6045*-associated *mecB* gene complex distinct from that of ΨSCC*mec*$_{KM0218}$. pKM0218 was almost identical to the *mecB*-containing plasmid recently reported in *Staphylococcus aureus* (overall 99.96% nucleotide identity). Mobilization of *mecB* within an unconventional circularizable structure was observed in Epi0076A as well as chromosomal plasmid insertion via recombination of *mecB* operons in KM0218. Our findings provide evidence of both the continuing evolution of *mecB*-containing elements in macrococci and *M. canis* as a potential source of the *mecB*-containing plasmid found in staphylococci.

### 2.2.6 García-Martín, A. B., Schwendener, S., Perreten, V., The *tva*(A) Gene from *Brachyspira hyodysenteriae* confers decreased susceptibility to pleuromutilins and streptogramin A in *Escherichia coli*. Antimicrob Agents Chemother 63 (2019).

*Abstract:* The *tva*(A) gene suspected to confer resistance to pleuromutilins in *Brachyspira hyodysenteriae* was tested for functionality in *Escherichia coli* AG100A and *Staphylococcus aureus* RN4220. Expression of the cloned *tva*(A) gene conferred decreased susceptibility to pleuromutilin (P) and streptogramin A ($S_A$) antibiotics in *E. coli* and had a minor effect in *S. aureus* The finding provides evidence of the direct association of *tva*(A) with the $PS_A$ resistance phenotype.

### 2.2.7 Hausherr, A., Becker, J., Meylan, M., Wuthrich, D., Collaud, A., Rossano, A.,

**Perreten, V., Antibiotic and quaternary ammonium compound resistance in *Escherichia coli* from calves at the beginning of the -fattening period in Switzerland (2017). Schweiz Arch Tierheilkd 161, 741-748 (2019).**

*Collaborators:* Clinic for Ruminants, Vetsuisse Faculty, University of Bern.

*Abstract:* In the Swiss veal calf production, antimicrobials and disinfectants are used to control bacterial infectious diseases, leading to a risk of selecting for a resistant bacterial population. While the prevalence of antibiotic resistance in *E. coli* from calves has been monitored at slaughterhouses in Switzerland since 2006, the resistance situation of *E. coli* from young calves entering the fattening period is not known. A total of 100 calves entering the fattening period in 20 geographically distant farms in Switzerland were screened for the presence of *E. coli* using rectal swabs in 2017. Genetic diversity between isolates was determined using repetitive palindromic Polymerase Chain Reaction (*rep*-PCR) revealing a genetically diverse *E. coli* population. Susceptibility to 13 antibiotics and to alkyldimethylbenzylammonium (ADBAC) was determined by the measurement of the minimal inhibitory concentration. Antibiotic and quaternary ammonium compound (QAC) resistance genes were identified using microarray and Polymerase Chain Reaction (PCR). Sixty-four percent of the isolates were resistant to at least one antibiotic, and 52% also exhibited decreased susceptibility to ADBAC. Resistance to more than 3 antibiotics was found in 40% of the isolates. Isolates exhibited resistance to tetracycline (57%) associated with the presence of *tet* genes (*tet*(A), (B), (E), (G)), to sulfonamides (61%) (*sul1*, *sul2*, *sul3*), ampicillin (56%) ($bla_{\text{TEM-1}}$), trimethoprim (32%) (*dfrA*), gentamicin (27%) (*ant(2'')-Ia*, *aac(3)-IVa*, *aac(3)-VIa*), and cefotaxime (2%) ($bla_{\text{CTX-M-14}}$ (ESBL)). Mutations in GyrA (S83L) and ParC (S80I) were found in the fluoroquinolone-resistant isolates (6%). All isolates were susceptible to colistin, tigecycline and meropenem. No association between the presence of decreased susceptibility to ADBAC and *qac* genes was observed. In conclusion, antibiotic and QAC resistant *E. coli* are present in the gastrointestinal tract of young calves at the beginning of the fattening period, emphasizing the need for appropriate and reduced use of antibiotics and QAC-containing disinfectants in order to limit further selection of these bacteria during the fattening period.

### 2.2.8 Hernandez-Fillor, R. E., Brilhante, M., Espinosa, I., Perreten, V., Complete circular genome sequence of a multidrug-resistant *Escherichia coli* strain from cuba obtained with Nanopore and Illumina hybrid assembly. Microbiol Resour Announc 8, e01269-19 (2019).

*Collaborators:* National Centre for Animal and Plant Health (CENSA), San José de las Lajas, Mayabeque, Cuba.

*Abstract:* The complete genome sequence of a multidrug-resistant *Escherichia coli* strain isolated from a healthy pig in Cuba was determined using short and long reads. This strain

carried four plasmids, including a 42,683-kb IncX1 plasmid, which contains the third-generation cephalosporin resistance gene *bla*$_{\text{CTX-M-32}}$ together with other disinfectant and antibiotic resistance genes.

### 2.2.9 Nigg, A., Brilhante, M., Dazio, V., Clement, M., Collaud, A., Gobeli Brawand, S., Willi, B., Endimiani, A., Schuller, S., Perreten, V., Shedding of OXA-181 carbapenemase-producing Escherichia coli from companion animals after hospitalisation in Switzerland: an outbreak in 2018. Euro Surveill 24 (2019).

*Collaborators:* Department of Clinical Veterinary Medicine, Bern, University of Bern; Institute for Infectious Diseases, University of Bern, Bern, Switzerland; Clinic for Small Animal Internal Medicine, University of Zurich, Zurich, Switzerland.

*Abstract:* Carbapenem-resistant *Enterobacteriaceae* pose a serious threat to public health worldwide, and the role of companion animals as a reservoir is still unclear. This 4-month prospective observational study evaluated carriage of carbapenem-resistant *Enterobacteriaceae* at admission and after hospitalisation in a large referral hospital for companion animals in Switzerland. Rectal swabs of dogs and cats expected to be hospitalised for at least 48 h were taken from May to August 2018 and analysed for the presence of carbapenem-resistant Enterobacteriaceae using selective agar plates. Resistant isolates were further characterised analysing whole genome sequences for resistance gene and plasmid identification, and ad hoc core genome multilocus sequence typing. This study revealed nosocomial acquisition of *Escherichia coli* harbouring the carbapenemase gene *bla*$_{\text{OXA-181}}$, the pAmpC cephalosporinase gene *bla*$_{\text{CMY-42}}$ as well as quinolone resistance associated with *qnrS1* and mutations in the topoisomerases II (GyrA) and IV (ParC). The *bla*$_{\text{OXA-181}}$ and *qnrS1* genes were identified on a 51 kb IncX3 plasmid and *bla*$_{\text{CMY-42}}$ on a 47 kb IncI1 plasmid. All isolates belonged to sequence type ST410 and were genetically highly related. This *E. coli* clone was detected in 17 of 100 dogs and four of 34 cats after hospitalisation (21.6%), only one of the tested animals having tested positive at admission (0.75%). Two positive animals were still carriers 4 months after hospital discharge, but were negative after 6 months. Companion animals may acquire carbapenemase-producing *E. coli* during hospitalisation, posing the risk of further dissemination to the animal and human population and to the environment.

### 2.2.10 Schwendener, S., Nigg, A., Collaud, A., Overesch, G., Kittl, S., Phumthanakorn, N., Perreten, V., Typing of *mecD* islands in genetically diverse methicillin-resistant *Macrococcus caseolyticus* strains from cattle. Appl Environ Microbiol 85 (2019).

*Collaborators:* Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

*Abstract: Macrococcus caseolyticus* belongs to the normal bacterial flora of dairy cows and does not usually cause disease. However, methicillin-resistant *M. caseolyticus* strains were isolated from bovine mastitis milk. These bacteria had acquired a chromosomal island (McRI$_{mecD}$-1 or McRI$_{mecD}$-2) carrying the methicillin resistance gene *mecD* To gain insight into the distribution of McRI$_{mecD}$ types in *M. caseolyticus* from cattle, 33 *mecD*-containing strains from Switzerland were characterized using molecular techniques, including multilocus sequence typing, antibiotic resistance gene identification, and PCR-based McRI$_{mecD}$ typing. In addition, the same genetic features were analyzed in 27 *mecD*-containing *M. caseolyticus* strains isolated from bovine bulk milk in England/Wales using publicly available whole-genome sequences. The 60 strains belonged to 24 different sequence types (STs), with strains belonging to ST5, ST6, ST21, and ST26 observed in both Switzerland and England/Wales. McRI$_{mecD}$-1 was found in different STs from Switzerland ($n$ = 19) and England/Wales ($n$ = 4). McRI$_{mecD}$-2 was only found in 7 strains from Switzerland, all of which belonged to ST6. A novel island, McRI$_{mecD}$-3, which contains a complete *mecD* operon (*mecD-mecR1*$_m$*-mecI*$_m$ [where the subscript *m* indicates *Macrococcus*]) combined with the left part of McRI$_{mecD}$-2 and the right part of McRI$_{mecD}$-1, was found in heterogeneous STs from both collections (Switzerland, $n$ = 7; England/Wales, $n$ = 21). Two strains from England/Wales carried a truncated McRI$_{mecD\text{-}3}$. Phylogenetic analyses revealed no clustering of strains according to geographical origin or carriage of McRI$_{mecD}$-1 and McRI$_{mecD}$-3. Circular excisions were also detected for McRI$_{mecD}$-1 and McRI$_{mecD}$-3 by PCR. The analyses indicate that these islands are mobile and may spread by horizontal gene transfer between genetically diverse *M. caseolyticus* strains.

### 2.2.11 Evaluation of EDTA- And DPA-based microdilution phenotypic tests for the detection of MCR-mediated colistin resistance in *Enterobacteriaceae*

*Collaborators:* Institute for Infectious Diseases, University of Bern, Bern, Switzerland; Clinical Microbiology and Virology Unit, A. Manzoni Hospital, Lecco, Italy.

*Abstract:* The emergence of the colistin-resistant (COL-R) Enterobacteriaceae represents a worrying health issue. However, only a portion of these strains may carry the plasmid-mediated *mcr* colistin resistance genes. We evaluated the ability of both ethylenediaminetetraacetic acid (EDTA)-based and dipicolinic acid (DPA)-based broth microdilution (BMD) tests to detect mcr-1 to mcr-5 producers. Of 92 Enterobacteriaceae (85 COL-R), 44 *mcr*-positive strains (39 *Escherichia coli*, 3 *Klebsiella pneumoniae*, and 2 *Salmonella* spp.) were tested. EDTA (100 µg/mL) was tested in Mueller-Hinton broth (MHB), whereas the DPA (900 µg/mL) was used in cation-adjusted MHB. Results were categorized as positive if in presence of chelator strains exhibited ≥3 two fold MIC decrease compared to the COL MIC alone. The EDTA-based BMD assay detected 41 *mcr*-positive strains, but 22 false-positive strains (including 12 *E. coli* and 4 *K. pneumoniae*) were recorded (sensitivity [SN], 93.2%; specificity [SP], 54.2%). The DPA-

based BMD assay detected 37 *mcr*-positive strains, with 7 false-negative (2 *E. coli*, 3 *K. pneumoniae*, 2 *Salmonella* spp.) strains (SN, 84.1%; SP, 100%). Overall, the EDTA-based BMD assay is not accurate to detect mcr producers, whereas the DPA-based BMD test ("colistin-MAC test") demonstrated good accuracy, but only when implemented for *E. coli* strains (SN, 94.9%; SP, 100%). With the aim to prevent the dissemination of *mcr*-possessing *E. coli* strains, the COL-MAC test could be implemented by clinical laboratories that are unable to perform molecular tests. Moreover, this assay could be applied to screen large collections of isolates to reveal the expression of new *mcr*-like genes not yet targeted by the current molecular assays.

### 2.2.12 Traversari, J., van den Borne, B. H. P., Dolder, C., Thomann, A., Perreten, V., Bodmer, M., Non-aureus staphylococci species in the teat canal and milk in four commercial Swiss dairy herds. Front Vet Sci 6, 186 (2019).

*Collaborators:* Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Bern, Switzerland; Business Economics Group, Wageningen University, Wageningen, Netherlands.

*Abstract:* Non-aureus staphylococci (NAS) are frequently found in milk samples as well as on the teat apex and in the teat canal and are known to be a cause of subclinical mastitis. The objective of this study was to investigate the relationship between NAS species colonizing the teat canal and those causing intramammary infection (IMI) in four commercial dairy herds. Teat canal swabs were obtained and thereafter milk samples were aseptically collected and evaluated for the presence of staphylococci using selective agar plates. Species identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The relationship between NAS species distribution and sample type (teat canal vs. milk samples) was quantified using hierarchical multivariable logistic regression models. The most prevalent NAS species in teat canal swabs were *S. xylosus* (35%), *S. vitulinus* (10%), and *S. chromogenes* (7%), whereas in milk samples *S. chromogenes* (5%), *S. xylosus* (5%), and *S. haemolyticus* (4%) were most prevalent. There were significantly higher odds for *S. vitulinus* (OR = 215), *S. xylosus* (OR = 20), *S. sciuri* (OR = 22), *S. equorum* (OR = 13), and *S. succinus* (OR = 10) to be present in teat canal swabs than in milk samples. Differences between herds in NAS species distribution were found and were most pronounced for *S. succinus* and a *S. warneri*-like species. This information aids in the understanding of NAS species as an etiology of IMI and should be taken into account when interpreting milk culture results.

### 2.2.13 van der Kolk, J. H., Endimiani, A., Graubner, C., Gerber, V., Perreten, V., *Acinetobacter* in veterinary medicine, with an emphasis on *Acinetobacter baumannii*. J Glob Antimicrob Resist 16, 59-71 (2019).

*Collaborators:* Swiss Institute of Equine Medicine (ISME), Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern and Agroscope, Länggassstrasse 124, 3012

Bern, Switzerland; Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, 3001 Bern, Switzerland.

*Abstract: Acinetobacter* spp. are aerobic, rod-shaped, Gram-negative bacteria belonging to the *Moraxellaceae* family of the class Gammaproteobacteria and are considered ubiquitous organisms. Among them, *Acinetobacter baumannii* is the most clinically significant species with an extraordinary ability to accumulate antimicrobial resistance and to survive in the hospital environment. Recent reports indicate that *A. baumannii* has also evolved into a veterinary nosocomial pathogen. Although *Acinetobacter* spp. can be identified to species level using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) coupled with an updated database, molecular techniques are still necessary for genotyping and determination of clonal lineages. It appears that the majority of infections due to *A. baumannii* in veterinary medicine are nosocomial. Such isolates have been associated with several types of infection such as canine pyoderma, feline necrotizing fasciitis, urinary tract infection, equine thrombophlebitis and lower respiratory tract infection, foal sepsis, pneumonia in mink, and cutaneous lesions in hybrid falcons. Given the potential multidrug resistance of *A. baumannii*, treatment of diseased animals is often supportive and should preferably be based on in vitro antimicrobial susceptibility testing results. It should be noted that animal isolates show high genetic diversity and are in general distinct in their sequence types and resistance patterns from those found in humans. However, it cannot be excluded that animals may occasionally play a role as a reservoir of *A. baumannii*. Thus, it is of importance to implement infection control measures in veterinary hospitals to avoid nosocomial outbreaks with multidrug-resistant *A. baumannii*.

### 2.2.14 Wüthrich, D., Brilhante, M., Hausherr, A., Becker, J., Meylan, M., Perreten, V., A Novel Trimethoprim Resistance Gene, *dfrA36*, Characterized from *Escherichia coli* from Calves. mSphere 4 (2019).

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

*Abstract:* Whole-genome sequencing of trimethoprim-resistant Escherichia coli strains MF2165 and PF9285 from healthy Swiss fattening calves revealed a so far uncharacterized dihydrofolate reductase gene, *dfrA36*. Functionality and association with trimethoprim resistance were demonstrated by cloning and expressing *dfrA36* in *E. coli*. The DfrA36 protein showed the closest amino acid identity (49.4%) to DfrA20 from *Pasteurella multocida* and to the Dfr determinants DfrG (41.2%), DfrD (40.8%), and DfrK (40.0%) found in Gram-positive bacteria. The *dfrA36* gene was integrated within a florfenicol/chloramphenicol-sulfonamide resistance IS*CR2* element (*floR*-IS*CR2*-*dfrA36*-*sul2*) next to a Tn*21*-like transposon that contained genes with resistance to sulfonamides (*sul1*), streptomycin (*aadA1*), gentamicin/tobramycin/kanamycin (*aadB*), and quaternary ammonium compounds (*qacE*Δ*1*).

A search of GenBank databases revealed that *dfrA36* was present in 26 other *E. coli* strains from different origins as well as in *Acinetobacter*.

# 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 analysed a total of 27816 samples. Details are shown in Table 1.

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

| Subdivision | Unit | Number of samples |
|---|---|---|
| Diagnostic Services and Epizootic Surveillance | Clinical material and mycology | 3186 |
| | Necropsy material, abortion and feaces | 2995 |
| | Molecular diagnostics (PCR incl. qPCR) | 1775 |
| | Bovine mastitis | 3041 |
| | Serology | 3065 |
| | Species identification | 239 |
| | Antibiograms for diagnostics | 2779 |
| Reference Laboratories and Resistance Monitoring | Antimicrobial resistance monitoring (detection) | 3837 |
| | Antimicrobial resistance monitoring (MIC*) | 6402 |
| | Reference laboratories | 497 |

* Minimal inhibitory concentration

## 3.1 Diagnostic Services and Epizootic Surveillance (Notifiable Animal Diseases)

Methods:

| | |
|---|---|
| Micr | Microscopic examination |
| IF | Immunofluorescence |
| 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 |
| LF | Antibody detection by lateral flow 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** | *Mycoplasma* | Cult | **Cattle** | 0 | 0 | 0 | 0 |
| **Pleuropneumonia** | *mycoides* subsp. | PCR | | 2 | 2 | 0 | 0 |
| | *mycoides* | ELISA | | 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** | 10 | 10 | 0 | 0 |
| | | Cult | | 10 | 10 | 0 | 0 |
| | *Bacillus anthracis* | Micr | **Pig** | 3 | 3 | 0 | 0 |
| | | Cult | | 0 | 0 | 0 | 0 |
| | *Bacillus anthracis* | Micr | **Sheep** | 7 | 5 | 2 | 0 |
| | | Cult | | 5 | 5 | 0 | 0 |
| **Brucellosis** | *Brucella abortus* | Micr | **Cattle** | 123 | 123 | 0 | 0 |
| | | RBT | | 35 | 32 | 0 | 3 |
| | | ELISA | | 804 | 801 | 0 | 3 |
| | | CFT | | 22 | 19 | 2* | 1 |
| | *Brucella melitensis* | Micr | **Sheep/goat** | 39 | 34 | 5 | 0 |
| | | ELISA | | 76 | 76 | 0 | 0 |
| | | CFT | | 0 | 0 | 0 | 0 |
| | | RBT | | 0 | 0 | 0 | 0 |
| | *Brucella abortus /* | Micr | **Others** | 42 | 41 | 1 | 0 |
| | *Brucella melitensis* | ELISA | | 569 | 553 | 3 | 13 |
| | | CFT | | 0 | 0 | 0 | 0 |
| | | RBT | | 60 | 60 | 0 | 0 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Brucellosis** | *Brucella suis* | Micr | **Pig** | 30 | 30 | 0 | 0 |
| | | RBT | | 644 | 643 | 0 | 1 |
| | | ELISA | | 1 | 1 | 0 | 0 |
| | * anticomplementär | CFT | | 70 | 62 | 8* | 0 |
| | *Brucella ovis* (epizootic to be controlled) | ELISA | **Sheep** | 31 | 30 | 0 | 1 |
| | *Brucella canis* (no epizootic) | PCR | **Dog** | 9 | 8 | 0 | 1 |
| | | Cult | | 4 | 4 | 0 | 0 |
| | | LF | | 39 | 28 | 0 | 11 |
| **Bovine genital Campylo-bacteriosis** | *Campylobacter fetus* subspecies *venerealis* | Cult | **Cattle** | 858 | 858 | 0 | 0 |
| | | PCR | | 34 | 34 | 0 | 0 |
| **Sproadic *Campylobacter* abortion** | *Campylobacter fetus* subspecies *fetus* (no epizootic) | Cult | **Ruminats** | 133 | 133 | 0 | 0 |
| **Glanders** | *Burkholderia mallei* | CFT | **Horse** | 11 | 7 | 1 | 3 |
| | | Cult | | 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 | **Cattle** | 558 | 558 | 0 | 0 |
| | | | **Dog** | 66 | 37 | 13 | 16 |
| | | | **Horse** | 14 | 11 | 0 | 3 |
| | | | **Pig** | 81 | 81 | 0 | 00 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Autumnalis | MAT | **Cattle** | 1 | 1 | 0 | 0 |
| | | | **Dog** | 65 | 51 | 10 | 4 |
| | | | **Horse** | 13 | 10 | 2 | 1 |
| | | | **Others** | 3 | 3 | 0 | 00 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Leptospirosis** | *L.* Ballum | MAT | **Cattle** | 65 | 65 | 0 | 0 |
| | | | **Dog** | 2 | 2 | 0 | 0 |
| | *L.* Ballum | MAT | **Pig** | 47 | 47 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Bataviae | MAT | **Cattle** | 1 | 1 | 0 | 0 |
| | | | **Dog** | 65 | 62 | 3 | 0 |
| | | | **Horse** | 4 | 4 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Bratislava | MAT | **Cattle** | 1 | 1 | 0 | 0 |
| | | | **Dog** | 66 | 40 | 6 | 20 |
| | | | **Horse** | 13 | 8 | 2 | 3 |
| | | | **Pig** | 49 | 49 | 0 | 0 |
| | | | **Sheep** | 14 | 14 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Canicola | MAT | **Cattle** | 557 | 557 | 0 | 0 |
| | | | **Dog** | 67 | 67 | 0 | 0 |
| | | | **Horse** | 13 | 13 | 0 | 0 |
| | | | **Pig** | 49 | 49 | 0 | 0 |
| | | | **Sheep** | 14 | 14 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Copenhageni | MAT | **Cattle** | 1 | 1 | 0 | 0 |
| | | | **Dog** | 58 | 42 | 10 | 6 |
| | | | **Others** | 3 | 3 | 0 | 00 |
| | *L.* Grippotyphosa | MAT | **Cattle** | 559 | 558 | 1 | 0 |
| | | | **Dog** | 65 | 55 | 7 | 3 |
| | | | **Horse** | 14 | 12 | 1 | 1 |
| | | | **Pig** | 49 | 48 | 1 | 0 |
| | | | **Sheep** | 14 | 14 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Hardjo | MAT | **Cattle** | 571 | 543 | 20 | 8 |
| | | | **Dog** | 65 | 65 | 0 | 0 |
| | | | **Horse** | 5 | 5 | 0 | 0 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Leptospirosis** | *L.* Hardjo | MAT | **Sheep** | 14 | 14 | 0 | 0 |
| | | | **Pig** | 5 | 5 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Icterohaemo-rrhagiae | MAT | **Cattle** | 558 | 558 | 0 | 0 |
| | | | **Dog** | 65 | 63 | 1 | 1 |
| | | | **Horse** | 14 | 14 | 0 | 0 |
| | | | **Pig** | 49 | 49 | 0 | 0 |
| | | | **Sheep** | 14 | 14 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Pomona | MAT | **Cattle** | 558 | 558 | 0 | 0 |
| | | | **Dog** | 65 | 57 | 8 | 0 |
| | | | **Horse** | 14 | 13 | 0 | 1 |
| | | | **Pig** | 55 | 52 | 1 | 0 |
| | | | **Sheep** | 14 | 14 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Pyrogenes | MAT | **Cattle** | 1 | 1 | 0 | 0 |
| | | | **Dog** | 65 | 64 | 1 | 0 |
| | | | **Horse** | 13 | 13 | 0 | 0 |
| | | | **Pig** | 2 | 2 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |
| | *L.* Sejroe | MAT | **Cattle** | 562 | 542 | 20 | 0 |
| | | | **Dog** | 10 | 10 | 0 | 0 |
| | | | **Horse** | 5 | 5 | 0 | 0 |
| | | | **Pig** | 2 | 2 | 0 | 0 |
| | | | **Others** | 2 | 2 | 0 | 0 |
| | *L.* Tarasosovi | MAT | **Cattle** | 65 | 65 | 0 | 0 |
| | | | **Dog** | 65 | 65 | 0 | 0 |
| | | | **Horse** | 14 | 14 | 0 | 0 |
| | | | **Pig** | 50 | 50 | 0 | 0 |
| | | | **Others** | 3 | 3 | 0 | 0 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Leptospirosis** | *Leptopsira* spp. | PCR | **Beaver** | 28 | 24 | 0 | 4 |
| | (pathogen) | | **Alpaca** | 6 | 6 | 0 | 0 |
| | | | **Pig** | 9 | 9 | 0 | 0 |
| | | | **Dog** | 11 | 5 | 0 | 6 |
| | | | **Horse** | 7 | 5 | 0 | 2 |
| | | | **Others** | 15 | 15 | 0 | 0 |
| **Salmonellosis** | *Salmonella* spp. | Cult/ST | **Alpaca** | 1 | 1 | | 0 |
| | | | **Ape** | 3 | 3 | | 0 |
| | | | **Bird** | 42 | 42 | | 0 |
| | | | **Cat** | 24 | 22 | | 2 |
| | *S.* Enteritidis | | | | | | 2 |
| | | | **Cattle** | 534 | 332 | | 202 |
| | *S.* Enteritidis | | | | | | 1 |
| | *S.* Typhimurium | | | | | | 5 |
| | *S.* Typhimurium, monophasic variant (4,12 : i : -) | | | | | | 196 |
| | | | **Chicken** | 3 | 3 | | 0 |
| | | | **Crocodile** | 1 | 1 | | 0 |
| | | | **Deer** | 1 | 1 | | 0 |
| | | | **Dog** | 45 | 42 | | 3 |
| | *S.* Typhimurium | | | | | | 1 |
| | *S.* Typhimurium, monophasic variant (4,12 : i : -) | | | | | | 2 |
| | | | **Fox** | 1 | 1 | | 0 |
| | | | **Fowl-like bird** | 12 | 11 | | 1 |
| | *S.* Enteritidis | | | | | | 1 |
| | | | **Goat** | 5 | 5 | | 0 |
| | | | **Goose** | 1 | 1 | | 0 |
| | | | **Horse** | 40 | 40 | | 0 |
| | | | **Lizard** | 20 | 8 | | 12 |
| **s** | *S.* Apapa* | | | | | | 1 |
| | *S.* Glosdrup | | | | | | 1 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Salmonellosis** | | | **Lizard** | | | | |
| | *S.* Muenchen | | | | | | 2 |
| | *S. enterica* subsp. *diarizonae* 65: z10: e,n,x,z15 | | | | | | 1 |
| | *S. enterica* subsp. *enterica* R: mt : - (Rauh-Form)* | | | | | | 1 |
| | *S. enterica* subsp. *enterica* R: z38 : - (Rauh-Form)* | | | | | | 1 |
| | *S. enterica* subsp. *houtenae* R: g,z51 : - (Rauh-Form)* | | | | | | 2 |
| | *S. enterica* subsp. *salamae* 58 : c : z6 | | | | | | 2 |
| | *S. enterica* subsp. *salamae* nicht bestimmbar* | | | | | | 1 |
| | | | **Monkey** | 13 | 13 | | 0 |
| | | | **Parrot** | 2 | 2 | | 0 |
| | | | **Pigeon** | 5 | 3 | | 2 |
| | *S.* Typhimurium, monophasic variant (4,12 : i : -) | | | | | | 2 |
| | | | **Rabbit** | 2 | 2 | | 0 |
| | | | **Sheep** | 13 | 11 | | 2 |
| | *S. enterica* subsp. *diarizonae* 61: k: 1,5,7 | | | | | | 2 |
| | | | **Snake** | 7 | 2 | | 5 |
| | *S. enterica* subsp. *arizonae* 21: z4,z23: - * | | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 61: i: z35* | | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 48: l,v: 1,5,7* | | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* R: l,v : - (Rauh-Form)* | | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* R: l,v : z35 (Rauh-Form)* | | | | | | 1 |
| | | | **Pig** | 31 | 24 | | 7 |
| | *S. enterica* subsp. *enterica* 9,12: - : - (O-Form)* | | | | | | 1 |
| | S. Infantis | | | | | | 3 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Salmonellosis** | *S.* Typhimurium, monophasic variant (4,12 : i : -) | | **Pig** | | | | 3 |
| | | | **Tortoise** | 2 | 2 | | 0 |
| | | | **Zoo animal** | 41 | 41 | | 0 |
| **Salmonellosis** | | | | **849** | | | **236** |
| **Contagious** | *Taylorella* | Cult | **Horse** | 279 | 279 | 0 | 0 |
| **equine metritis** | *equigenitalis* | | **Donkey** | 6 | 6 | 0 | 0 |
| **Enzootic pneumonia in swine** | *Mycoplasma hyopneumoniae* | PCR Lung (pooled) | **Pig** | 97 | 92 | 0 | 5 |
| | | PCR Nasal swabs (pooled) | | 50 | 46 | 0 | 4 |
| | | PCR Projec (single)t | | 207 | 160 | 0 | 47 |
| | | ELISA | | 91 | 47 | 1 | 43 |
| **Swine actinobacillosis** | *Actinobacillus pleuropneumoniae* | Cult/PCR | **Pig** | 243 | 227 | 0 | 16 |
| | I BD +II CA, Serotyp 7,12 | | | | | | 10 |
| | I BD+II CA+III CA+BD, Serotyp 2 | | | | | | 4 |
| | I BD + II CA, Serotyp 2 | | | | | | 2 |
| | II CA+IIICA+BD, Serotyp 3 | | | | | | |
| | | ELISA ApxIV | | 2 | 0 | 0 | 2 |

### 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** | *C. jejuni / C. coli* | Cult | **Dog** | 42 | 36 | 2 | 4 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Campylobac-teriosis** | *C. jejuni / C. coli* | | **Cat** | 21 | 19 | 1 | 1 |
| | | | **Others** | 59 | 57 | 1 | 1 |
| **Listeriosis** | *Listeria monocytogenes* | Cult | **Ruminants** | 12 | 8 | 0 | 4 |
| | | | **Primates** | 8 | 4 | 0 | 0 |
| | | | **Others** | 9 | 6 | 0 | 3 |
| **Yersiniosis** | *Yersinia/ enterocolitica* | Cult | **Monkey** | 16 | 16 | 0 | 0 |
| | | | **Cattle** | 1 | 0 | 0 | 1 |
| | | | **Others** | 23 | 23 | 0 | 0 |
| | *Yersinia pseudotuberculosis* | Cult | **Hare** | 10 | 7 | 0 | 3 |
| | | | **Others** | 27 | 27 | 0 | 0 |
| **Caseous lymphadenitis in sheep/goat** | *Corynebacterium pseudotuberculosis (ovis)* | Cult | **Goat** | 1 | 1 | 0 | 0 |
| | | | **Sheep** | 2 | 1 | 0 | 1 |
| **Enzootic abortion in ewes (chlamydiosis)** | *Chlamydia abortus* | Micr | **Sheep** | 12 | 11 | 1 | 0 |
| | | ELISA | | 47 | 47 | 0 | 0 |
| | | PCR | | 0 | 0 | 0 | 0 |
| | | Micr | **Goat** | 25 | 21 | 4 | 0 |
| | | ELISA | | 4 | 2 | 0 | 2 |
| | | PCR | | 4 | 4 | 0 | 0 |
| | | Micr | **Cattle** | 18 | 18 | 0 | 0 |
| | | ELISA | | 0 | 0 | 0 | 0 |
| | | PCR | | 5 | 5 | 0 | 0 |
| | | Micr | **Others** | 28 | 28 | 0 | 0 |
| | | ELISA | | 3 | 3 | 0 | 0 |
| | | PCR | | 12 | 12 | 0 | 0 |
| **Psittacosis** | *Chlamydia psittaci* | PCR | **Bird** | 17 | 15 | 0 | 2 |
| | | Micr | | 28 | 28 | 0 | 0 |
| **Tularaemia** | *Francisella tularensis* | Cult/PCR | **Hare** | 50 | 22 | 0 | 28 |
| | | | **Others** | 23 | 18 | 0 | 5 |
| **Blackleg** | *Clostridium chauvoei* | IF | **Ruminant** | 9 | 9 | 0 | 0 |
| | | Cult | | 10 | 8 | 0 | 2 |

| Epizootic | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Coxiellosis** | *Coxiella burnetii* | Micr | **Cattle** | 123 | 123 | 0 | 0 |
| | | ELISA | | 24 | 19 | 0 | 5 |
| | | PCR | | 6 | 6 | 0 | 0 |
| | | Micr | **Sheep** | 12 | 11 | 1 | 0 |
| | | ELISA | | 33 | 32 | 0 | 1 |
| | | PCR | **Sheep** | 1 | 0 | 0 | 1 |
| | | Micr | **Goat** | 25 | 21 | 4 | 0 |
| | | ELISA | | 6 | 3 | 0 | 3 |
| | | PCR | | 13 | 5 | 0 | 8 |
| | | Micr | **Others** | 28 | 27 | 1 | 0 |
| | | ELISA | | 1 | 1 | 0 | 0 |
| | | PCR | | 12 | 6 | 0 | 6 |

### 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* | 30 | 29 | 0 | 1 |
| | | | *virulent D.nodosus* | 30 | 12 | 0 | 18 |
| | PCR (single or pool) | **Cattle** | *benigne D.nodosus* | 2 | 1 | 0 | 1 |
| | | | *Virulent D.nodosus* | 2 | 2 | 0 | 0 |
| | PCR (single or pool) | **Goat** | *benigne D.nodosus* | 2 | 2 | 0 | 0 |
| | | | *Virulent D.nodosus* | 2 | 2 | 0 | 0 |

## 3.2 Reference Laboratories and Resistance Monitoring

### 3.2.1 Antimicrobial Resistance Monitoring

Program for food producing animals from Switzerland and meat at retail

The program follows the specifications laid down in the decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria in Europe. Caecal samples from pigs and calves were collected at slaughter and cultured for indicator *E. coli*, *Campylobacter* spp. (excl. calves), extended spectrum beta-lactamases (ESBLs) and carbapenemases producing *E. coli*. Moreover, fresh meat thereof from retail was analysed for ESBLs, carbapenemases producing *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Isolated strains and all *Salmonella enterica* subspecies *enterica* strains from diagnostics and reference function were tested for antimicrobial susceptibility. Testing was performed by applying the Minimal Inhibitory Concentration (MIC) method.

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

The numbers of analyses that are part of the 2019 antimicrobial resistance monitoring are displayed in tables 6a to 6f.

Table 6a: Number of analyses on Methicillin-resistant *S. aureus* (MRSA)

| Matrix | Number of analyses |
|---|---|
| beef meat samples | 309 |
| porcine meat samples | 311 |
| **Total** | **620** |

Table 6b: Number of analyses on ESBL/pAmpC producing *E. coli*

| Matrix | Number of analyses |
|---|---|
| calves caecal samples | 298 |
| beef meat samples | 309 |
| porcine caecal samples | 306 |
| porcine meat samples | 311 |
| **Total** | **1224** |

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

| Matrix | Number of analyses |
|---|---|
| calves caecal samples | 298 |
| beef meat samples | 309 |
| porcine caecal samples | 306 |
| porcine meat samples | 311 |
| **Total** | **1224** |

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

| Matrix | Number of analyses |
|---|---|
| calves caecal samples | 212 |
| porcine caecal samples | 207 |
| **Total** | **419** |

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

| Matrix | Number of analyses |
|---|---|
| porcine caecal samples | 350 |
| **Total** | **350** |

Table 6f: Number of analyses on *Salmonella enterica* subspecies *enterica*

| Salmonella serovar | Number of analyses |
|---|---|
| *S.* Enteritidis | 14 |
| *S.* Typhimurium | 41 |
| *S.* Typhimurium, monophasic variant | 11 |
| other serovars | 41 |
| **Total** | **107** |

### 3.2.2 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 | **Cattle** | 0 | 0 | 0 | 0 |
| | Cult | | 0 | 0 | 0 | 0 |
| **Bovine brucellosis** | ELISA | **Cattle** | 13 | 6 | 1 | 6 |
| | RBT | | 13 | 13 | 0 | 0 |
| | CFT | | 13 | 8 | 4 | 1 |
| | CULT | | 0 | 0 | 0 | 0 |
| | PCR | | 0 | 0 | 0 | 0 |
| **Caprine and ovine** | ELISA | **Sheep/ goat** | 0 | 0 | 0 | 0 |
| **brucellosis** | RBT | | 0 | 0 | 0 | 0 |
| | CFT | | 0 | 0 | 0 | 0 |
| **Porcine brucellosis** | ELISA | **Swine** | 0 | 0 | 0 | 0 |
| | RBT | | 0 | 0 | 0 | 0 |
| | CFT | | 0 | 0 | 0 | 0 |
| | CULT | | 0 | 0 | 0 | 0 |
| **Canine brucellosis** | LF | **Dog** | 0 | 0 | 0 | 0 |
| | Micr | | 0 | 0 | 0 | 0 |
| | Cult | | 0 | 0 | 0 | 0 |
| | Direct PCR | | 0 | 0 | 0 | 0 |

| Epizootic | Method | Animal | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|
| **Ovine epididymitis (*Brucella ovis*)** | ELISA | **Sheep** | 0 | 0 | 0 | 0 |
| **Bovine genital campylobacteriosis** | CULT | **Cattle** | 2 | 2 | 0 | 0 |
| **Swine actinobacillosis** | ELISA ApxIV | **Swine** | 0 | 0 | 0 | 0 |
| | Cult | | 36 | 2 | 0 | 34 |
| | PCR | | 34 | 0 | 0 | 34 |
| **Contagious equine metritis** | Cult | **Horse** | 5 | 1 | 0 | 4 |
| | PCR | | 0 | 0 | 0 | 0 |
| **Blackleg** | IF | **Cattle** | 0 | 0 | 0 | 0 |
| | Cult | | 0 | 0 | 0 | 0 |
| | PCR | | 2 | 2 | 0 | 0 |
| **Campylobacteriosis** | ID | **Dog** | 2 | 0 | 0 | 2 |
| | | **Cattle** | 2 | 1 | 0 | 1 |
| | | **Poultry** | 22 | 19 | 0 | 3 |
| **Enzootic pneumonia in pig (incl. EP Diagnostic evaluation)** | ELISA | **Swine** | | | | |
| | PCR Dubosson | | 20 | 2 | 0 | 18 |
| | PCR Strait | | | | | |
| | | | 45 | 30 | 0 | 15 |
| | | | 46 | 30 | 0 | 16 |
| **Tularaemia** | ID | **Hare** | 1 | 0 | 0 | 1 |
| **Yersiniosis** | ID | **Fish** | 0 | 0 | 0 | 0 |
| **Antimicrobial resistance** | ID, MIC | **Diverse** | 21 | 0 | 0 | 21 |

### 3.2.3 Serotyping of *Salmonella* sp.

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

| Serovar | Animal | Number |
|---|---|---|
| *S*. Abony | Cattle | 1 |
| *S*. Abortusovis | Sheep | 2 |
| | Goat | 1 |
| *S*. Agona | Chicken | 3 |
| | Quail | 1 |
| *S*. Ago* | Lizard | 3 |
| *S*. Albany | Chicken | 6 |
| | Turkey | 8 |
| *S*. Apapa* | Lizard | 1 |
| *S*. Bardo | Horse | 1 |
| *S*. Brandenburg | Pig | 1 |
| *S*. Bredeney | Pig | 1 |
| *S*. Cerro* | Lizard | 1 |
| *S*. Dublin | Cattle | 1 |
| *S*. Eboko | Horse | 1 |
| *S*. Enteritidis | Cattle | 5 |
| | Chicken | 10 |
| | Laying hen | 2 |
| | Duck | 2 |
| | Partridge | 1 |
| | Dog | 1 |
| | Cat | 2 |
| | Horse | 3 |
| | Laying hen | 3 |
| | Hedgehog | 3 |
| *S*. Glosdrup | Reptil | 1 |
| *S*. Heidelberg | Chicken | 1 |
| *S*. Hessarek | Laying hen | 1 |
| *S*. Irumu | Lizard | 1 |
| *S*. Kenntucky | Chicken | 1 |
| *S*. Kisarawe | Lizard | 2 |
| *S*. Livingstone | Chicken | 1 |

| Serovar | Animal | Number |
|---|---|---|
| *S*. Mbandaka | Chicken | 1 |
| *S*. Mikawasima | Chicken | 1 |
| | Laying hen | 2 |
| *S*. Newport | Snake | 1 |
| *S*. Schleissheim | Cattle | 1 |
| *S*. Tennessee | Chicken | 4 |
| | Laying hen | 1 |
| | Lizard | 2 |
| *S*. Typhimurium | Cattle | 24 |
| | Chicken | 7 |
| | Laying hen | 9 |
| | Turkey | 3 |
| | Cat | 1 |
| | Horse | 4 |
| | Rabbit | 7 |
| *S*. Typhimurium, monophasic variant (4,12 : i : -) | Cattle | 6 |
| | Laying hen | 2 |
| | Chicken | 6 |
| | Horse | 1 |
| | Pig | 2 |
| *S*. Veneziana | Laying hen | 1 |
| *S. enterica* subsp. *arizonae* 21: z4,z23: -* | Snake | 1 |
| *S. enterica* subsp. *arizonae* 21: g,z51: -* | Snake | 1 |
| *S. enterica subsp. diarizonae 48: i: z** | Chicken | 1 |
| *S. enterica subsp. diarizonae 48: l,v: 1,5,7** | Lizard | 1 |
| *S. enterica subsp. diarizonae 53: k: e,n,x,z15* | Lizard | 1 |
| *S. enterica subsp. diarizonae 53: z10: z35** | Lizard | 2 |
| *S. enterica subsp. diarizonae 58: r: z35** | Snake | 1 |
| *S. enterica subsp. diarizonae 61: k: 1,5,7* | Sheep | 9 |
| *S. enterica subsp. enterica 9,12: - : - (O-Form)** | Pig | 1 |
| *S. enterica subsp. enterica 13,23:i : - (Rauh-Form)** | Dog | 1 |
| *S. enterica subsp. houtenae 11: z4,z23 : -* | Snake | 1 |
| *S. enterica subsp. houtenae 44: z4,z23 : -* | Lizard | 1 |

| Serovar | Animal | Number |
|---|---|---|
| *S. enterica* subsp. *salamae* 47 : b : e,n,x,z15* | Lizard | 1 |
| *S. enterica* subsp. *salamae* 58 : c : z6 | Lizard | 1 |
| *S. enterica* subsp. *salamae* nicht bestimmbar* | Lizard | 1 |
| No *Salmonella* | | 2 |
| | **Total** | **184** |

* serotyping in human reference laboratory

### 3.2.4 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 | 20 |
| Biovar I | apx group: I BD + II CA + III CA + BD<br>*cps2* gene positive | 2 | 2 |
| Biovar I | apx group: I CA + BD +II CA | 1,5,9,11 | 1 |
| Biovar I | apx group: II CA + III CA + BD | 3 | 7 |
| Biovar I | apx group: III CA + BD<br>*other variant of serotype 3* | 3 | 2 |
| Biovar II | apx group: I BD + II CA<br>*cps2* gene positive | 2 | 2 |
| No APP | - | - | 2 |
| | | **Total** | **36** |

### 3.2.5 Leptospirosis Diagnostics for Humans

Table 10: Number of samples investigated related to humane medicine

| Zoonosis | Agent | Method | Host | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|---|
| **Leptospirosis** | *L.* Andaman | MAT | **Human** | 5 | 5 | 0 | 0 |
| | *L.* Australis | | | 5 | 3 | 0 | 2 |
| | *L.* Autumnalis | | | 5 | 4 | 0 | 1 |
| | *L.* Ballum | | | 5 | 5 | 0 | 0 |
| | *L.* Bataviae | | | 5 | 5 | 0 | 0 |
| | *L.* Bratislava | | | 5 | 3 | 0 | 2 |
| | *L.* Canicola | | | 5 | 5 | 0 | 0 |
| | *L.* Celledoni | | | 4 | 4 | 0 | 0 |
| | *L.* Copenhageni | | | 5 | 4 | 1 | 0 |
| | *L.* Cynopetri | | | 5 | 4 | 1 | 0 |
| | *L.* Grippotyphosa | | | 5 | 4 | 0 | 1 |
| | *L.* Hardjo | | | 5 | 0 | 0 | 0 |
| **Leptospirosis** | *L.* Hebdomalis | MAT | **Human** | 5 | 5 | 0 | 0 |
| | *L.* Icterohaemo-rrhagiae | | | 5 | 5 | 0 | 0 |
| | *L.* Javanica | | | 3 | 3 | 0 | 0 |
| | *L.* Panama | | | 4 | 4 | 0 | 0 |
| | *L.* Patoc | | | 5 | 4 | 1 | 0 |
| | *L.* Pomona | | | 5 | 5 | 0 | 0 |
| | *L.* Pyrogenes | | | 5 | 5 | 0 | 0 |
| | *L.* Sejroe | | | 4 | 4 | 0 | 0 |
| | *L.* Shermani | | | 4 | 4 | 0 | 0 |
| | *L.* Tarassovi | | | 5 | 4 | 1 | 0 |
| | pathogene Leptospiren | PCR | **Human** | 18 | 13 | 0 | 5 |

### 3.2.6 Organisation of Proficiency Testing for approved laboratories

As Swiss national reference laboratory for anthrax, brucellosis, salmonellosis 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 for these laboratories, which are mandatory for the approval by the Federal Food Safety and Veterinary Office. The proficiency testings conducted in 2019 are listed in Table 11.

Table 11: Proficiency testings for approved laboratories organised by the ZOBA in 2019

| Target | Method | Number of samples | Number of laboratories |
|---|---|---|---|
| Antimicrobial susceptibility testing | Diverse | 6 | 11 |
| Questionaire Anthrax microscopy | - | - | 9 |
| Salmonellosis | Serotyping methods | 10 | 10 |

## 3.3 Research Activities

### 3.3.1 Gobeli Brawand, S., Keller, C., Overesch, G., Outbreak of salmonellosis in cattle caused by the unusual *Salmonella* serotype Stockholm. *Veterinary Record Case Reports* 7, 1-5 (2019).

*Collaborators:* Cantonal Veterinary Service Bern, Bern, Switzerland

*Abstract*: Salmonellosis in livestock is not only a problem for farmers due to economic losses but is also a human health concern because of its zoonotic nature. In Europe, bovine enteric salmonellosis is known to be caused by a limited number of serotypes, that is, *Salmonella enterica* subspecies *enterica* (*S.*) serotypes Typhimurium and Dublin. Here, we describe an outbreak of salmonellosis in a Swiss cattle herd caused by *S.* Stockholm. To the authors' knowledge, in cattle, this serotype has hitherto only been described once: isolated from beef cattle in a slaughterhouse in India. On the other hand, *S.* Stockholm has been isolated at least once from the stool of a patient suffering from gastroenteritis (Kantele 2011). This outbreak demonstrates that all known non-typhoidal *S enterica* subspecies *enterica* serotypes, despite their rare detection, have to be considered pathogenic and potentially zoonotic agents.

### 3.3.2 Schonecker, L., Schnyder, P., Overesch, G., Schupbach-Regula, G., Meylan, M., Associations between antimicrobial treatment modalities and antimicrobial susceptibility in *Pasteurellaceae* and *E. coli* isolated from veal calves under field

**conditions. *Vet Microbiol*** 236**, 108363 (2019).**

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

*Abstract*: Antimicrobial consumption, with bovine respiratory disease as main indication, is higher in the veal calf industry compared to other livestock production branches. The aim of the present study was to investigate possible associations between antimicrobial drug use and resistance in *Pasteurellaceae* and indicator *Escherichia* (*E*.) *coli* from veal calves under field conditions in a prospective trial. Over a period of one year, nasopharyngeal and rectal swabs were collected from 2587 animals on 12 and 43 farms, respectively. Antimicrobial susceptibility testing was performed on 346 *Mannheimia* (*M*.) *haemolytica*, 1,162 *Pasteurella* (*P*.) *multocida* and 2,138 *E. coli*. Drug use was quantified as treatment incidence for each farm based on the used daily dose methodology (TIUDD), separately for group and individual treatments, and for antimicrobial classes. In multivariable mixed logistic regression analyses, risk factors could be identified for reduced susceptibility to certain antimicrobial classes. Group treatment was generally associated with higher rates of not susceptible (NS) *M. haemolytica* and *P. multocida a*nd non-wildtype (non-WT) *E. coli*. Individual treatment was associated with less NS and non-WT isolates. Age and entry protocol were important confounders with younger animals showing higher rates of NS and non-WT strains. The present findings suggest that, under field conditions, targeted individual treatment of calves can reduce the development of antimicrobial resistance compared to oral group treatment. For the different microorganisms, risk factors for resistance were partially different. This demonstrates that indicator organisms like *E. coli* do not necessarily reflect the associations observed in respiratory pathogens.

### 3.3.3 Gobeli Brawand, S., Kittl, S., Dettwiler, M., Thomann, A., Feyer, S., Cachim, J., Theubet, G., Liechti, N., Wittwer, M., Schurch, N., Oberhansli, S., Heinimann, A., Jores, J., An unusual case of bovine anthrax in the canton of Jura, Switzerland in 2017. BMC Vet Res 15, 265 (2019).

*Collaborators:* Institute of Animal Pathology, University of Bern, Bern, Switzerland; Animal Health, General Management of Agriculture, Viticulture and Veterinary Affairs (DGAV), Canton of Vaud, Switzerland; Veterinaires Mont-Terri Sarl, Courgenay, Switzerland; Spiez Laboratory, Federal Office for Civil Protection, Spiez, Switzerland; Interfaculty Bioinformatics Unit, University of Bern, Bern, Switzerland; Institute of Geography and Centre for Development and Environment, University of Bern, Bern, Switzerland.

*Abstract:* BACKGROUND: Anthrax caused by *Bacillus anthracis* is a zoonotic disease mainly affecting herbivores. The last Swiss outbreak was over 20 years ago. We describe a recent anthrax outbreak involving two cows from the same herd. One cow was designated as a peracute clinical case with sudden death and typical lung lesions, while the other cow presented with protracted fever and abortion.

CASE PRESENTATION: On April 29th 2017, a 3.5-year-old Montbeliard dairy cow was found dead while out at pasture with haemorrhage from the nose. The veterinarian suspected pneumonia and performed a necropsy on site. Subsequently, a lung and liver sample were sent to the laboratory. Unexpectedly, *Bacillus anthracis* was isolated, a pathogen not found in Switzerland for decades. Several days later, a second cow from the same farm showed signs of abortion after protracted fever. Since these symptoms are not typical for anthrax, and the bacteria could not be demonstrated in blood samples from this animal, a necropsy was performed under appropriate biosafety measures. Subsequently, *Bacillus anthracis* could be isolated from the placenta and the sublumbal lymph nodes but not from the blood, liver, spleen and kidney. The outbreak strain (17OD930) was shown to belong to the lineage B.Br.CNEVA, the same as Swiss strains from previous outbreaks in the region. We speculate that the disease came from a temporarily opened cave system that is connected to an old carcass burial site and was flushed by heavy rainfall preceding the outbreak.
CONCLUSION: Even in countries like Switzerland, where anthrax is very rare, new cases can occur after unusual weather conditions or ground disturbance. It is important for public officials to be aware of this risk to avoid possible spread.

# 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 |

## 4.3 Clinical Topics

| | |
|---|---|
| Population Medicine: | 8 x 45 min |

## 4.4 Hands on Courses

| | |
|---|---|
| Practical Course in Bacteriology: | 48 x 45 min |
| Practical Course in Microbial and Immunological Diagnostics: | 56 x 45 min |

# 5 Publications

## 5.1 Peer-Reviewed Publications


1. Akarsu, H., Aguilar-Bultet, L., Falquet, L., deltaRpkm: an R package for a rapid detection of differential gene presence between related bacterial genomes. *BMC Bioinformatics* **20**, 621 (2019).
2. Albert, E., Biksi, I., Nemet, Z., Csuka, E., Kelemen, B., Morvay, F., Bakos, Z., Bodo, G., Toth, B., Collaud, A., Rossano, A., Perreten, V., Outbreaks of a methicillin-resistant *Staphylococcus aureus* clone ST398-t011 in a Hungarian equine clinic: Emergence of rifampicin and chloramphenicol resistance after treatment with these antibiotics. *Microb Drug Resist* **25**, 1219-1226 (2019).
3. Alinaitwe, L., Kankya, C., Allan, K. J., Rodriguez-Campos, S., Torgerson, P., Dreyfus, A., Bovine leptospirosis in abattoirs in Uganda: Molecular detection and risk of exposure among workers. *Zoonoses Public Health* **66**, 636-646 (2019).
4. Alsaaod, M., Locher, I., Jores, J., Grimm, P., Brodard, I., Steiner, A., Kuhnert, P., Detection of specific *Treponema* species and *Dichelobacter nodosus* from digital dermatitis (Mortellaro's disease) lesions in Swiss cattle. *Schweiz Arch Tierheilkd* **161**, 207-215 (2019).
5. Baby, V., Labroussaa, F., Lartigue, C., Rodrigue, S., Synthetic chromosomes: rewriting the code of life. *Med Sci (Paris)* **35**, 753-760 (2019).
6. Balestrin, E., Kuhnert, P., Wolf, J. M., Wolf, L. M., Fonseca, A. S. K., Ikuta, N., Lunge, V. R., Siqueira, F. M., Clonality of *Mycoplasma hyopneumoniae* in swine farms from Brazil. *Vet Microbiol* **238**, 108434 (2019).
7. Baschera, M., Cernela, N., Stevens, M. J. A., Liljander, A., Jores, J., Corman, V. M., Nuesch-Inderbinen, M., Stephan, R., Shiga toxin-producing *Escherichia coli* (STEC) isolated from fecal samples of African dromedary camels. *One Health* **7**, 100087 (2019).
8. Brilhante, M., Perreten, V., Donà, V., Multidrug resistance and multivirulence plasmids in enterotoxigenic and hybrid Shiga toxin-producing/enterotoxigenic *Escherichia coli* isolated from diarrheic pigs in Switzerland. *Vet J* **244**, 60-68 (2019).
9. Brilhante, M., Donà, V., Overesch, G., Endimiani, A., Perreten, V., Characterisation of a porcine *Escherichia coli* strain from Switzerland carrying *mcr-1* on a conjugative multidrug resistance IncHI2 plasmid. J Glob Antimicrob Resist **16**, 123-124 (2019)
10. Büdel, T., Clément, M., Bernasconi, O.J., Principe, L., Perreten, V., Luzzaro, F., Endimiani, A., Evaluation of EDTA- and DPA-based microdilution phenotypic tests for

the detection of MCR-mediated colistin resistance in *Enterobacteriaceae*. Microb Drug Resist **25**, 494-500 (2019).

11. Chanchaithong, P., Perreten, V., Am-In, N., Lugsomya, K., Tummaruk, P., Prapasarakul, N., Molecular Characterization and Antimicrobial Resistance of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* Isolates from Pigs and Swine Workers in Central Thailand. *Microb Drug Resist* **25**, 1382-1389 (2019).
12. Chanchaithong, P., Perreten, V., Schwendener, S., *Macrococcus canis* contains recombinogenic methicillin resistance elements and the *mecB* plasmid found in *Staphylococcus aureus*. *J Antimicrob Chemother* **74**, 2531-2536 (2019).
13. García-Martín, A. B., Schwendener, S., Perreten, V., The *tva*(A) gene from *Brachyspira hyodysenteriae* confers decreased susceptibility to pleuromutilins and streptogramin A in *Escherichia coli*. *Antimicrob Agents Chemother* **63** (2019).
14. Gobeli Brawand, S., Keller, C., Overesch, G., Outbreak of salmonellosis in cattle caused by the unusual *Salmonella* serotype Stockholm. *Veterinary Record Case Reports* **7**, 1-5 (2019).
15. Gobeli Brawand, S., Kittl, S., Dettwiler, M., Thomann, A., Feyer, S., Cachim, J., Theubet, G., Liechti, N., Wittwer, M., Schurch, N., Oberhansli, S., Heinimann, A., Jores, J., An unusual case of bovine anthrax in the canton of Jura, Switzerland in 2017. *BMC Vet Res* **15**, 265 (2019).
16. Hausherr, A., Becker, J., Meylan, M., Wuthrich, D., Collaud, A., Rossano, A., Perreten, V., Antibiotic and quaternary ammonium compound resistance in *Escherichia coli* from calves at the beginning of the -fattening period in Switzerland (2017). *Schweiz Arch Tierheilkd* **161**, 741-748 (2019).
17. Hernandez-Fillor, R. E., Brilhante, M., Espinosa, I., Perreten, V., complete circular genome sequence of a multidrug-resistant *Escherichia coli* strain from Cuba obtained with Nanopore and Illumina Hybrid assembly. *Microbiol Resour Announc* **8** (2019).
18. Jores, J., Ma, L., Ssajjakambwe, P., Schieck, E., Liljander, A., Chandran, S., Stoffel, M. H., Cippa, V., Arfi, Y., Assad-Garcia, N., Falquet, L., Sirand-Pugnet, P., Blanchard, A., Lartigue, C., Posthaus, H., Labroussaa, F., Vashee, S., Removal of a Subset of Non-essential Genes Fully Attenuates a Highly Virulent *Mycoplasma* Strain. *Front Microbiol* **10**, 664 (2019).
19. Jores, J., Schieck, E., Liljander, A., Sacchini, F., Posthaus, H., Lartigue, C., Blanchard, A., Labroussaa, F., Vashee, S., *In vivo* role of capsular polysaccharide in *Mycoplasma mycoides*. *J Infect Dis* **219**, 1559-1563 (2019).

20. Josi, C., Burki, S., Vidal, S., Dordet-Frisoni, E., Citti, C., Falquet, L., Pilo, P., Large-Scale Analysis of the *Mycoplasma bovis* Genome Identified Non-essential, Adhesion- and Virulence-Related Genes. *Front Microbiol* **10**, 2085 (2019).
21. Kuhnert, P., Cippa, V., Hardi-Landerer, M. C., Schmicke, M., Ulbrich, S., Locher, I., Steiner, A., Jores, J., Early Infection Dynamics of *Dichelobacter nodosus* During an Ovine Experimental Footrot In Contact Infection. *Schweiz Arch Tierheilkd* **161**, 465-472 (2019).
22. Labroussaa, F., Baby, V., Rodrigue, S., Lartigue, C., Whole genome transplantation: bringing natural or synthetic bacterial genomes back to life. *Med Sci (Paris)* **35**, 761-770 (2019).
23. Lartigue, C., Valverde Timana, Y., Labroussaa, F., Schieck, E., Liljander, A., Sacchini, F., Posthaus, H., Batailler, B., Sirand-Pugnet, P., Vashee, S., Jores, J., Blanchard, A., Attenuation of a Pathogenic *Mycoplasma* Strain by Modification of the *obg* Gene by Using Synthetic Biology Approaches. *mSphere* **4** (2019).
24. Liljander, A., Sacchini, F., Stoffel, M. H., Schieck, E., Stokar-Regenscheit, N., Labroussaa, F., Heller, M., Salt, J., Frey, J., Falquet, L., Goovaerts, D., Jores, J., Reproduction of contagious caprine pleuropneumonia reveals the ability of convalescent sera to reduce hydrogen peroxide production in vitro. *Vet Res* **50**, 10 (2019).
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28. Salt, J., Jores, J., Labroussaa, F., Wako, D. D., Kairu-Wanyoike, S. W., Nene, V., Stuke, K., Mulongo, M., Sirand-Pugnet, P., Vaccination against CCPP in East Africa. *Vet Rec* **185**, 272 (2019).
29. Schonecker, L., Schnyder, P., Overesch, G., Schupbach-Regula, G., Meylan, M., Associations between antimicrobial treatment modalities and antimicrobial

susceptibility in *Pasteurellaceae* and *E. coli* isolated from veal calves under field conditions. *Vet Microbiol* **236**, 108363 (2019).

30. Schumacher, M., Nicholson, P., Stoffel, M. H., Chandran, S., D'Mello, A., Ma, L., Vashee, S., Jores, J., Labroussaa, F., Evidence for the Cytoplasmic Localization of the L-alpha-Glycerophosphate Oxidase in Members of the "*Mycoplasma mycoides* Cluster". *Front Microbiol* **10**, 1344 (2019).
31. Schwendener, S., Nigg, A., Collaud, A., Overesch, G., Kittl, S., Phumthanakorn, N., Perreten, V., Typing of *mecD* Islands in Genetically Diverse Methicillin-Resistant *Macrococcus caseolyticus* Strains from Cattle. *Appl Environ Microbiol* **85** (2019).
32. Traversari, J., van den Borne, B. H. P., Dolder, C., Thomann, A., Perreten, V., Bodmer, M., Non-aureus Staphylococci Species in the Teat Canal and Milk in Four Commercial Swiss Dairy Herds. *Front Vet Sci* **6**, 186 (2019).
33. Trueeb, B. S., Gerber, S., Maes, D., Gharib, W. H., Kuhnert, P., Tn-sequencing of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* mutant libraries reveals non-essential genes of porcine mycoplasmas differing in pathogenicity. *Vet Res* **50**, 55 (2019).
34. van der Kolk, J. H., Endimiani, A., Graubner, C., Gerber, V., Perreten, V., *Acinetobacter* in veterinary medicine, with an emphasis on *Acinetobacter baumannii*. *J Glob Antimicrob Resist* **16**, 59-71 (2019).
35. Weldearegay, Y. B., Muller, S., Hanske, J., Schulze, A., Kostka, A., Ruger, N., Hewicker-Trautwein, M., Brehm, R., Valentin-Weigand, P., Kammerer, R., Jores, J., Meens, J., Host-Pathogen Interactions of *Mycoplasma mycoides* in Caprine and Bovine Precision-Cut Lung Slices (PCLS) Models. *Pathogens* **8** (2019).
36. Wüthrich, D., Brilhante, M., Hausherr, A., Becker, J., Meylan, M., Perreten, V., A Novel Trimethoprim Resistance Gene, *dfrA36*, Characterized from *Escherichia coli* from Calves. *mSphere* **4** (2019).

### 5.2 Book Chapters

- Kuhnert, P. *Basfia* In: *Bergey's Manual of Systematics of Archaea and Bacteria*, ed W.B. Whitman, John Wiley, Chichester, UK, 2019

### 5.3 Other Publications

- ARCH-Vet Bericht 2018 Bericht über den Vertrieb von Antibiotika und Antibiotikaresistenzen in der Veterinärmedizin in der Schweiz

*Main authors:* Dagmar Heim, Federal Food Safety and Veterinary Office, and Gudrun Overesch, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern

- Clostridieninfektionen beim Kleinwiederkäuer, Forum Kleinwiederkäuer 11/2019

  *Author:* Lutz Schönecker, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern

## 5.4 Press Releases and Broadcasting

- "Antimicrobial resistant bacteria in shrimps" TV broadcast SRF1, "Kassensturz", 12.02.2019, Gudrun Overesch, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern

# 6 Graduations and Visiting Scientists

## 6.1 PhD Degrees

Name of student: Bettina Trüeb

Title of the Thesis: Host-pathogen interaction and underlying genetics of porcine mycoplasmas

Supervisor. Peter Kuhnert

Abstract: *Mycoplasma hyopneumoniae* is the etiological agent of porcine enzootic pneumonia; a disease characterized by dry cough and reduced weight gain. This species is closely related to *Mycoplasma hyorhinis*, which colonizes the tonsils and nasal cavity of healthy pigs but can also be isolated from arthritic joints and polyserositis. Both species cause economic losses in the pig industry and no effective vaccines preventing colonization are available today. Similar to most mycoplasma species, the nature of the disease caused is chronic rather than acute indicating that the immune system cannot effectively remove the bacteria from the organism. In a first study, *M. hyorhinis* isolates from clinical cases in Switzerland and Germany were genotyped to identify possible virulent clones. Similar to a previous observation with *M. hyopneumoniae*, there was a limited clonality with predominant genotypes found in a single animal and at the farm level, however, with no specific genotypes or clusters associated with clinical manifestations like pneumonia, arthritis or polyserositis. Thus the two mycoplasma species show similar genetic variability with no specific virulent clones, however they differ in their pathogenicity. Currently, only few virulence associated genes are known for *M. hyopneumoniae* and *M. hyorhinis* and their differences in pathogenicity remain unsolved despite the genome sequences of both having been explored. We therefore aimed to identify possible virulence associated genes that might also act as the driver of the disparities in pathogenicity. For this purpose, field isolates for both species were genome-sequenced and transposon mutant libraries generated. For the *M. hyopneumoniae* strain F7.2C and the *M. hyorhinis* strain JF5820, the non-essential and species-specific gene sets were determined through sequencing their transposon-mutant libraries and through bidirectional Blastp analysis. For both strains, sets of species-specific non-essential genes include known and possibly new virulence associated genes as well as large numbers of hypothetical protein coding sequences. Finally, we investigated the innate immune response towards the two mycoplasma species. Porcine PBMCs were stimulated with live and inactivated mycoplasmas. Both mycoplasma species induced a strong TNF response in monocytes. They also induced a strong upregulation of CD40 in particular in monocytes and also in conventional dendritic cells 1. Mycoplasmas activated $IgM^+$ B cells including both the $CD21^+$ naïve and innate-like $CD21^-$ subset in terms of CD25 upregulation, proliferation, and IgM secretion. CD25 was higher with *M. hyorhinis* while IgM secretion was much higher following stimulation with *M. hyopneumoniae*. Overall, inactivated mycoplasmas stimulate in a reduced manner compared to live bacteria. In conclusion, we have determined the individual non-essential genes of two porcine mycoplasmas being of interest in the development of an attenuated live vaccine and provide a comparative analysis of innate immune response with different porcine antigen presenting cells.

## 6.2 Dr. vet. med. Degrees

Name of student: Lutz Schönecker

Title of Thesis: Associations between antimicrobial treatment modalities and antimicrobial susceptibility in *Pasteurellaceae* and *E. coli* isolated from veal calves under field conditions.

Supervisors: Gudrun Overesch (Institute of Veterinary Bacteriology,University of Bern, Switzerland), Mirelle Meylan (Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland), Gertraud Schüpbach (Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Switzerland)

Abstract: Antimicrobial consumption, with bovine respiratory disease as main indication, is higher in the veal calf industry compared to other livestock production branches. The aim of the present study was to investigate possible associations between antimicrobial drug use and resistance in Pasteurellaceae and indicator *Escherichia* (*E.*) *coli* from veal calves under field conditions in a prospective trial. Over a period of one year, nasopharyngeal and rectal swabs were collected from 2,587 animals on 12 and 43 farms, respectively. Antimicrobial susceptibility testing was performed on 346 *Mannheimia* (*M.*) *haemolytica*, 1,162 *Pasteurella* (*P.*) *multocida* and 2,138 *E. coli*. Drug use was quantified as treatment incidence for each farm based on the used daily dose methodology (TIUDD), separately for group and individual treatments, and for antimicrobial classes. In multivariable mixed logistic regression analyses, risk factors could be identified for reduced susceptibility to certain antimicrobial classes. Group treatment was generally associated with higher rates of not susceptible (NS) *M. haemolytica* and *P. multocida* and non-wildtype (non-WT) *E. coli*. Individual treatment was associated with less NS and non-WT isolates. Age and entry protocol were important confounders with younger animals showing higher rates of NS and non-WT strains. The present findings suggest that, under field conditions, targeted individual treatment of calves can reduce the development of antimicrobial resistance compared to oral group treatment. For the different microorganisms, risk factors for resistance were partially different. This demonstrates that indicator organisms like *E. coli* do not necessarily reflect the associations observed in respiratory pathogens.

Name of student: Melanie Edith Schumacher

Title of Thesis: Work towards the localization of *Mycoplasma* proteins suggested to be involved in virulence.

Supervisors: Jörg Jores (Institute of Veterinary Bacteriology,University of Bern, Switzerland), Michael Stoffel

Abstract: *Mycoplasma* (*M.*) of the "*M. mycoides* cluster" are causative agents for several livestock diseases. L-α-glycerophosphate oxidase (GlpO) is a candidate virulence factor of *M. mycoides*. It is a central enzyme in the metabolism of glycerol, producing $H_2O_2$ as a by-product. The current model that GlpO is surface-localized has been disproved in this work. The

screening of all species of the "*M. mycoides* cluster" using Triton X-114 partitioning and immunoblotting has clearly shown that the GlpO is located in the cytoplasm. In addition, scanning electron microscopy experiments based on the M. mycoides subsp. capri deletion mutant GM12::YCpMmyc1.1-*ΔglpO* showed unspecific binding of the anti-GlpO antibody used previously to claim surface-localization of GlpO.

Another virulence factor in *M. mycoides* is capsular polysaccharide. Additionally, *M. mycoides* glycosylates many proteins. There are four Cps- and EpsG-glycosyltransferases encoding genes annotated in the genome of GM12, which are candidates for transferring sugar moieties to proteins/lipids or to link them to other sugars. Testing knock out mutants for glycosylation would give information about their role in that procedure. Using Coomassie and glycoprotein staining comperatively, no difference was detected in between different mutants. Moreover the Cps-glycosyltransferases have been located in the cytoplasma using Triton X-114 partitioning and immunoblotting whereas the EpsG-glycosyltransferases were below the detection limit.

Name of student: Flavio Sacchini

Title of Thesis: Establishment and use of novel challenge models for caprine *Mycoplasma* of the '*M. mycoides* cluster' to investigate host-pathogen interactions

Supervisors: Jörg Jores (Institute of Veterinary Bacteriology,University of Bern, Switzerland)

Part 1: Reproduction of contagious caprine pleuropneumonia reveals the ability of convalescent sera to reduce hydrogen peroxide production in vitro

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae* is an important livestock disease that is widespread in the Middle East, Asia and Africa. The infection is acquired through inhalation of contaminated droplets and can cause 100% morbidity and up to 80% mortality. Limited knowledge is available on the pathogenesis of this organism, mainly due to the lack of a robust *in vivo* challenge model and the means to do site-directed mutagenesis. This work describes the establishment of a novel caprine challenge model for CCPP that resulted in 100% morbidity using a combination of repeated intranasal spray infection followed by a single transtracheal infection employing the recent Kenyan outbreak strain ILRI181. Diseased animals displayed CCPP-related pathology and the bacteria could subsequently be isolated from pleural exudates and lung tissues in concentrations of up to $10^9$ bacteria per mL as well as in the trachea using immunohistochemistry. Reannotation of the genome sequence of ILRI181 and $F38^T$ revealed the existence of genes encoding the complete glycerol uptake and metabolic pathways involved in hydrogen peroxide ($H_2O_2$) production in the phylogenetically related pathogen *M. mycoides* subsp. *mycoides*. Furthermore, the expression of L-α-glycerophosphate oxidase (GlpO) *in vivo* was confirmed. In addition, the function of the glycerol metabolism was verified by measurement of production

of $H_2O_2$ in medium containing physiological serum concentrations of glycerol. Peroxide production could be inhibited with serum from convalescent animals. These results will pave the way for a better understanding of host–pathogen interactions during CCPP and subsequent vaccine development.

Part 2: In vivo role of capsular polysaccharide in *Mycoplasma mycoides*

Capsular polysaccharides (CPSs) have been confirmed to be an important virulence trait in many gram-positive and gram-negative bacteria. Similarly, they are proposed to be virulence traits in minimal *Mycoplasma* that cause disease in humans and animals. CPSs are at the interface between the bacterium and its host and constitute the first line of defense against the immune system. In the current study, we tested whether the CPS of *M. mycoides* constitutes a virulence trait. Using a caprine challenge model, we compared the outcomes of experimental infections with a highly virulent *M. mycoides* subsp. *capri* strain (GM12::YCpMmyc1.1) or its CPS-lacking mutant derivative (GM12::YCpMmyc1.1-*Δglf*), lacking the capsular polysaccharide, galactofuranose. Goats were infected with either strain, and clinical parameters and pathomorphological changes were evaluated. Goats infected with the mutant strain showed only transient fever. In contrast, 5 of 8 goats infected with the parental strain reached end-point criteria after infection. Attenuation was shown for GM12::YCpMmyc1.1-*Δglf*, demonstrating a role of CPS as a virulence factor for this important group of mycoplasmas.

Name of student: Aurélien Nigg

Title of Thesis: Shedding of OXA-181-carbapenemase-producing Escherichia coli from companion animals after hospitalization in Switzerland, 2018.

Supervisor: Vincent Perreten (Institute of Veterinary Bacteriology, University of Bern, Switzerland)

Abstract: Carbapenem-resistant *Enterobacteriaceae* pose a serious threat to public health worldwide, and the role of companion animals as a reservoir is still unclear. This surveillance study evaluated carriage of carbapenem-resistant *Enterobacteriaceae* at admission and after hospitalization in a companion animal clinic in Switzerland and revealed nosocomial acquisition of *E. coli* harbouring the carbapenemase gene $bla_{\text{OXA-181}}$, the pAmpC cephalosporinase gene $bla_{\text{CMY-42}}$, as well as quinolone resistance associated with *qnrS1* and mutations in the topoisomerases II (GyrA) and IV (ParC). Whole genome sequence analysis identified the $bla_{\text{OXA-181}}$ and *qnrS1* genes on a 51-kb IncX3 plasmid and the $bla_{\text{CMY-42}}$ on a 47-kb IncI1 plasmid. All isolates belonged to sequence type ST410 and were genetically highly related as determined by an ad-hoc core genome multilocus sequence typing (cgMLST) scheme. This *E. coli* clone was detected in 17 dogs and 4 cats after hospitalization (21.6%), only one of them having tested positive at admission (0.75%). Two of the positive animals were still carriers up to four months after hospital discharge, but were negative after six months. This

study showed that companion animals may acquire carbapenemase-producing *E. coli* during hospitalization, posing the risk of further dissemination to the animal and human population and to the environment.

Name of student: Dominik Wüthrich.

Title of Thesis: A novel trimethoprim resistance gene *dfrL* characterized from *Escherichia coli* from calves.

Supervisor: Vincent Perreten (Institute of Veterinary Bacteriology, University of Bern, Switzerland)

Abstract: Whole-genome sequencing of trimethoprim-resistant *Escherichia coli* strains MF2165 and PF9285 from healthy Swiss fattening calves revealed a so far uncharacterized dihydrofolate reductase gene *dfrL*. Functionality and association with trimethoprim resistance was demonstrated by cloning and expressing *dfrL* in *E. coli*. The DfrL protein showed the closest amino acid identity (50.0%) to DfrA20 from *Pasteurella multocida* and to the Dfr determinants DfrD (42.6%), DfrG (42.9%), DfrK (41.7%) found in Gram-positive bacteria. The *dfrL* gene was integrated into the chromosome on a large 43-kb element, which also contained genes with resistance to sulfonamides (*sul1, sul2*), florfenicol/chloramphenicol (*floR*), streptomycin (*aadA1*), gentamicin/tobramycin/kanamycin (*aadB*) and quaternary ammonium (*qacEΔ1*). Searching GenBank databases revealed that *dfrL* was present in 26 other *E. coli* from different origins as well as in *Acinetobacter*.

The presence of *dfrL* in *E. coli* from animals as well as its presence in other *E. coli* from different sources and countries and in *Acineobacter*, highlights the global spread of this gene and its potential for dissemination. The genetic link of *dfrL* with other antibiotic and disinfectant resistance genes showed that multidrug-resistant *E. coli* may be selected and maintained by the use of either one of several antimicrobials.

### 6.3 Master Degrees

Name of student: Sarah Moser (Master thesis in Veterinary Medicine)

Title of Thesis: Antibiotic resistance of feacal *E.coli* in Swiss veal calves operations.

Supervisor: Gudrun Overesch (Institute of Veterinary Bacteriology,University of Bern, Switzerland)

Name of student: Manuela Luginbühl (Master thesis in Veterinary Medicine)

Title of Thesis: Septic Peritonitis

Supervisors: Cristina Pérez Vera, (Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern) and Gudrun Overesch (Institute of Veterinary Bacteriology,University of Bern, Switzerland)

Name of student: Javier Eduardo Fernandez (Master thesis in Molecular Life Sciences)

Title of Thesis: Novel macrolide resistance genes in *Macrococcus canis*

Supervisors: Vincent Perreten, Sybille Schwendener (Institute of Veterinary Bacteriology,University of Bern, Switzerland)

### 6.4 Visiting Scientists

Rosa Elena Hernández Fillor. Characterization of 3rd generation cephalosporin-resistant *Escherichia coli* isolated from pigs in Cuba using Next Generation Sequencing. National Centre for Animal and Plant Health (CENSA), San José de las Lajas, Mayabeque, Cuba.

Alongkorn Kurilung. Genomic characterization and pan-genome analysis of *Leptospira weilii* isolates from asymptomatic dogs. Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

# 7 Scientific Meetings Organized, Keynotes given and Grants Awarded

## 7.1 Scientific Meetings Organized by IVB Staff

- Jörg Jores, Scientific program committee member of the European Mycoplasma Conference, London (UK), March 18-19, 2019

## 7.2 Keynote/Invited Lectures Given by IVB Staff

- Gudrun Overesch, 31th January 2019 Rothenburg, Switzerland, Title: Antibiotikaresistenzen: Resistenzsituation in der Veterinärmedizin (Nutztiere – Lebensmittel – Heimtiere). Fortbildungveranstaltung der Gesellschaft Zentral Scheizerischer Tierärzte (GZST)
- Peter Kuhnert, 18th March 2019, Colindale, London, UK, Title: Genotypic monitoring of *Mycoplasma hyopneumoniae* reveals imported pigs as an additional risk factor for recurrent cases of enzootic pneumonia in Switzerland. European Mycoplasma Conference
- Peter Kuhnert, 25th April 2019, Bern, Switzerland, Title: Atuelle Labordiagnostik. Moderhinke Bekämpfung in der Schweiz – ein Update. Fortbildung für Tierärztinnen und Tierärzte
- Gudrun Overesch, 26th April 2019 Kgs. Lyngby, Denmark, Title: MRSA prevalence in Swiss slaughter pigs since 2009. 13th Annual Workshop of the European Reference Laboratory for Antimicrobial Resistance
- Vincent Perreten. 6th – 10th May 2019. Use of next generation sequencing (NGS) for antibiotic resistance surveillance 3rd International Seminar on Animal and Plant Health (SISA 2019) and the 20th Congress of the Latin American Phytopathological Association, Varadero, Cuba.
- Vincent Perreten. 16th May 2019.Zwanzig Jahre Forschung über Antibiotika-Resistenzen *„Twenty years of technology advancement for the identification of antibiotic resistance”*. Veranstaltung der Alumni Vetsuisse-Fakultät Bern, Bern.
- Vincent Perreten. 21st May 2019. Carbapenemase producing *Enterobacteriaceae* in animals. One Health meets Sequencing – KKL Lucerne, Switzerland.
- Gudrun Overesch, 29th May 2019 Amersfoort, Netherlands, Title: Activities of the NRL - Salmonella Switzerland. 24th workshop of the European Reference Laboratory for Salmonella
- Vincent Perreten. 1st - 3rd July 2019. Discovery of novel antibiotic resistance determinants using next-generation sequencing. 8th Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE 2019), Vinci International Convention Centre, Tours Val de Loire – France, Keynote lecture.
- Gudrun Overesch, 07th November 2019, Parma, Italy, Title: Antimicrobial resistance in Swiss *Salmonella* isolates. Scientific Network for Zoonoses Monitoring Data, 9th specific meeting on Antimicrobial Resistance data reporting by the European Food Safety Authority (EFSA)

## 7.3 Competitive Grants Awarded

- Donor: Swiss National Science Foundation (SNSF). Resistome in the pig farms: Comparison of the breeding and fattening units with a One Health approach. Duration: 12.2018 - 12.2021. Principal Investigator: Markus Hilty (Institute for Infectious Diseases, University of Bern, Switzerland); Co-applicants: Gudrun Overesch (Institute of Veterinary Bacteriology, University of Bern, Switzerland), Anne Oppliger (Institute for Work and Health, University of Lausanne, Switzerland).
- Donor: Federal Food Safety and Veterinary Office (FSVO). Therapeutic guide for antimicrobial stewardship in exotic pets. Duration: 10.2019 - 9/2020. Principal Investigator: Jean-Michel Hatt (Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Switzerland); Co-applicants: Gudrun Overesch (Institute of Veterinary Bacteriology, University of Bern, Switzerland); Hanspeter Naegeli (Institute of Veterinary Pharmacology and Toxicology, University of Zurich, Switzerland).
- Donor: SNSF and Innosuisse – Swiss Innovation Agency. Microbial Epimerases: A Toolbox for the Synthesis of Novel Peptide-Based Drugs. BRIDGE joint programme project no. 40B2-0_180993. Principal Investigator. R. Buller (ZHAW School of Life Sciences and Facility Management, Wädenswil); Co-applicants: V. Perreten (Institute of Veterinary Bacteriology, University of Bern, Switzerland), R. Bruggmann (Interfaculty Bioinformatics Unit, Interfaculty Bioinformatics Unit.
- Donor: Federal Food Safety and Veterinary Office (FSVO). Molecular epidemiology of *Brachyspira hyodysenteriae* in the Swiss pig production. FSVO Project no. 1.19.05. Principal Investigator: V. Perreten (Institute of Veterinary Bacteriology, University of Bern, Switzerland); 1st co-applicant: F. Zeeh, Clinic for Swine, University of Bern, Switzerland).

## 7.4 Other funding

- Donor: Federal Food Safety and Veterinary Office (FSVO). Monitoring of antimicrobial resistance in veterinary pathogens from clinical admissions. Duration: 01.2019 – 12.2021. Pricipal Investigator: Gudrun Overesch (Institute of Veterinary Bacteriology, University of Bern, Switzerland)).

# 8 Organization Chart (Organigram)