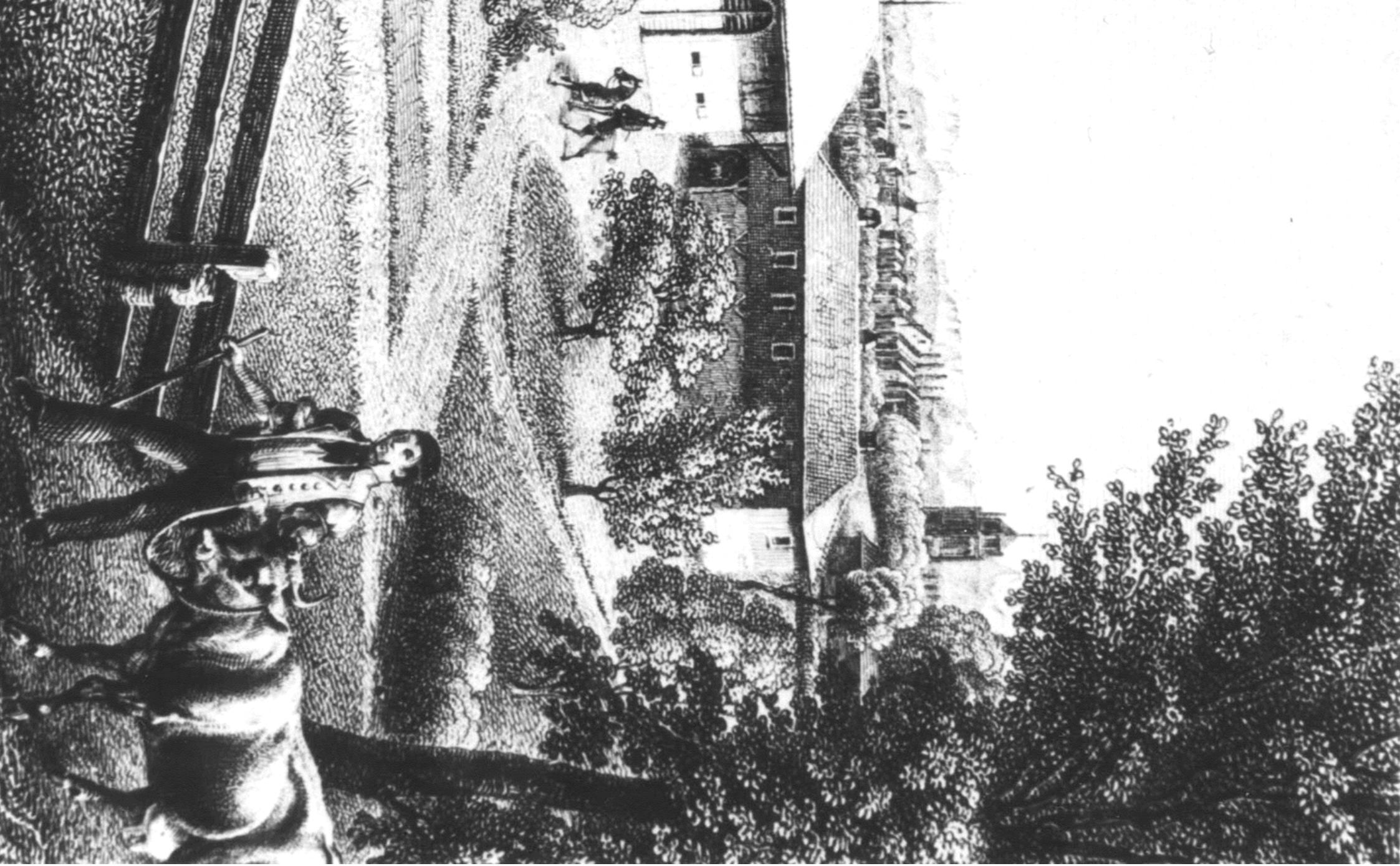

Jahresbericht - Annual Report 2015
Research, ZOBA
Institut für Veterinär-Bakteriologie, Vetsuisse Fakultät Universität Bern

UNIVERSITÄT BERN

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## Contents

## 1. Preface

The present annual report gives a short overview on the highlight activities of the Institute during the year 2015. The scientific output was particularly beneficial as revealed by the large number of publications and collaborative studies. Major foci during 2015 represented the NF Sinergia Project on Comparative study of human and ruminant neurolisteriosis as a clue to understand *Listeria monocytogenes* neurotropism, discoveries of several novel antibiotic resistance genes as well as several surveillance- and applied research-projects that were done in the framework of the Institute's duties as National Centre for Zoonotic and Enzootic Pathogens and Antibiotic Resistance.

The fruitful contributions in the field of basic research on molecular pathogenicity and applied molecular diagnostics of *Dichelobacter nodosus,* the etiological agent of ovine footrot, that constitutes a central tool in the control of this disease, led to a successful parliamentarian intervention in 2015 that finalized in a new law to control and eradicate footrot in Switzerland.

The large number of invitations of scientists from the Institute of Veterinary Bacteriology as experts and guest speakers on many international and national congresses dealing with the topics of bacterial pathogenesis and antibiotic resistance testified for the international expertise and quality of our research projects.

In order to fulfil our duties in continuous education, the residency program for the preparation to become Diplomate of the American College of Veterinary Microbiology (ACVM) was opened to the whole Vetsuisse Faculty of the Universities Bern and Zürich and currently accounts for 6 residents that are trained in Veterinary Microbiology.

Bern, 31 March 2016 Joachim Frey

Cover: The Veterinary School Bern from 1826 – 1890. Engraving of Rubeli 1906

## 2. Research Units

### 2.1. Pathobiology and Molecular Mechanisms of Bacterial Virulence

#### 2.1.1. A novel *Porphyromonas* species, *'Porphyromonas katsikii*' causing pyogranulomatous pneumonia in goats

Joachim Frey
Collaborative study with Dr. George Filioussis, Dr. Evanthia Petridou and Dr. Emmanouel Karavanis, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece.

*Porphyromonas* species belonging to the phylum *Bacteroidetes* are non-motile, Gram-negative, rod-shaped, anaerobic bacteria that are considered as emerging pathogens in both humans and animals. In general, *Porphyromonas* species, and in particular *Porphyromonas gingivalis*, the best studied representative of the genus, are known as oral pathogens causing gingivitis, periodontitis, endodontic diseases and apical abscesses in human and animals. However, several *Porphyromonas* species are involved in other human and animal pathologies such as metritis, peritonitis, interdigital necrobacillosis and necrotic vulvovaginitis. Given that phenotypic identification and discrimination of *Porphyromonas* and *Prevotella* species has been shown to give inconsistent results, genotypic species identification based on 16S rRNA gene sequences was developed by our laboratory previously and had been used successfully thereafter.

An outbreak of acute respiratory disease affecting 6 out of 25 goats occurred in a small farm in the northwest of Greece. The animals showed loss of appetite for 3 days. The clinical examination revealed high fever (41.5$^{o}$C), lethargy, an empty-appearing abdomen, hyperactive rumen motility, purulent nasal discharge, open-mouth breathing and tachypnea. Microscopic examination of Giemsa-stained, thin and thick blood films did not reveal the presence of any blood parasites (*Anaplasma ovis, Babesia ovis* and *Babesia motasi*). The animals were treated intramuscularly with 20 mg/kg of tylosin (Tylan 50, Elanco) twice a day which did not result in clinical improvement all 6 affected animals died a few days of the therapy. The remaining animals showed no symptoms. There were no risk factors such as stress, crowding or viral infections recorded for the herd prior to the outbreak. In the affected animals, gross lesions were confined to the lungs and consisted of generalized interstitial edema with multiple light tan to light yellow branching and anastomosing tracts. The edema were moderately elevating the surface of the whole lung parenchyma. They were consistent with severely ectatic airways frequently distended to form prominent nodular collection of exudate ranging from 1 mm to 4 cm in diameter. Morphologically these lesions are consistent with a sub-acute, severe, and multifocal to coalescing bronchopneumonia with severe bronchiectasis presumably of infectious origin.

Microbiological examination of lung samples taken from the multiple ectatic airways lumina revealed no growth on all conditions tested after 96 h. However, after 10 – 14 days growth under anaerobic conditions, small black pigmented colonies of rod shaped Gram-negative bacteria were found on blood – agar medium from samples of 3 affected animals. These bacteria also grew on blood – agar medium supplemented with Kanamycin or Ampicillin, but not with Vancomycin. DNA amplification from the black pigmented colonies and subsequent sequence analysis of the amplified *rrs* 16S rRNA gene showed the same 1408 bp sequence for isolates of all 3 samples (Gen Bank accession nr. KM 360064). Comparison of this DNA

sequence by BLAST revealed 92% sequence similarity (identical nt) with the *rrs* gene of *Porphyromonas levii* (Gen Bank accession nr. L16493) and *Porphyromonas somerae* (Gen Bank accession nr. L16493) as the closest known bacterial species.
*Porphyromonas somerae* and *Porphyromonas levii*, two closely related species are regarded as pathogenic species of human and cattle, respectively. Clinical manifestations of *P. somerae* (*P. levii*–like) include soft-tissue and bone infections, brain abscesses, and otitis media with mastoiditis. In ruminants, *P. levii* has been isolated from bovine necrobacillosis, papillomatous digital dermatitis, and acute interdigital phlegmon among cows, as well as from an outbreak of bovine necrotic vulvovaginitis. However *P. levii* was also reported as an opportunistic pathogen in the rumen of cattle and was also found in healthy cattle herds.

The *Porphyromonas* species isolated from the lung of the diseased goats were tentatively named '*Porphyromonas katsikii'* (κατσίκα gr. goat). PCR amplification with specific primers for this yet undescribed species revealed the presence '*Porphyromonas katsikii'* in lung tissue of all affected animals while no PCR signals were evidenced from lungs of healthy goats or from goats with pasteurellosis caused by *Mannheimia haemolytica*. These data indicate '*Porphyromonas katsikii'* to be the causative agent of acute respiratory distress.

Filioussis, G., Petridou, E., Karavanis, E. and Frey, J. (2015) Pyogranulomatous pneumonia in goats caused by an undescribed *Porphyromonas* species: '*Porphyromonas katsikii*'. J. Clin. Microbiol. **53**:795-798.

## 2.1.2. Pathobiology of *Mycoplasma mycoides* subsp. *mycoides*, the etiologic agent of CBPP

Joachim Frey
Collaborative study with Dr. Jörg Jores, and Dr. Jan Naessens, International Livestock Research Institute ILRI, Nairobi Kenya and Dr. Johann Weber, Center for integrative Genomics, University of Lausanne and Swiss Institute for Bioinformatics, Lausanne, Switzerland.

Members of the '*Mycoplasma mycoides* cluster' represent important livestock pathogens worldwide*. Mycoplasma mycoides* subsp. *mycoides* is the etiologic agent of contagious bovine pleuropneumonia (CBPP), which is still endemic in many parts of Africa. Contagious bovine pleuropneumonia (CBPP) is a serious respiratory disease of cattle characterized by coughing, fever and respiratory distress, and results in major economic losses particularly in sub-Saharan Africa. Whereas CBPP has been controlled in a number of developed countries through a combination of co-ordinated surveillance, eradication of whole infected herds and quarantine, these methods are not effective in developing countries due to geopolitical, economic and cultural considerations. Vaccination is therefore considered the most appropriate control measure for CBPP in sub-Saharan Africa. However, the most widely deployed vaccine against CBPP in sub-Saharan Africa, as recommended by FAO, is the live attenuated *M. mycoides* subsp. *mycoides* vaccine strain T1/44, which induces only short-lived immunity and causes severe post-vaccinal skin lesions in a proportion of vaccinates. In addition, there is evidence that that CBPP-associated lung damage has a significant immunological component. Surface lipoproteins have been identified as potential triggers of the massive inflammatory reaction that is caused by *M. mycoides* subsp. *mycoides* and other mycoplasmal infections. Whereas the role of mycoplasma lipoproteins in induction of pro-inflammatory

cytokines has been demonstrated data are limited as to the precise role of these proteins in the outcome of disease. The *M. mycoides* subsp. *mycoides* trans-membrane Lipoprotein Q (LppQ) has been shown to induce strong, early and persistent antibody responses in cattle undergoing natural CBPP infection. This led to the development of a CBPP diagnostic ELISA based on the membrane-exposed N terminal fragment of LppQ (LppQ-N'). A vaccine trial using LppQ-N′ formulated in Freund's adjuvant was carried out in cattle. The clinical, pathological and immunological outcome after challenge was evaluated with an infective dose of virulent *M. mycoides* subsp. *mycoides*. Vaccinated animals revealed a strong seroconversion to LppQ, but they exhibited exacerbated disease upon challenge with a virulent strain B237 of *M. mycoides* subsp. *mycoides*, as evidenced by more severe clinical, gross pathological, histological and immuno-histological outcomes. Features of enhanced pathology included lung vasculitis and glomerulonephritis, consistent with involvement of antigen-antibody immune complexes. These findings reveal the pathogenesis of enhanced disease as a result of antibodies against LppQ present at challenge and argue against inclusion of LppQ-N′ in a future subunit vaccine for CBPP. Based on our observations, we describe a novel mechanism pathogenesis of CBPP.

In order to get a better genetic basis for future research on the pathobiology *M. mycoides* subsp. *mycoides* the genome sequences and annotation of two frequently used challenge strains of *M. mycoides* subsp. *mycoides*, strain Afadé and strain B237 were completed. The information will enable downstream 'omics' applications such as proteomics, transcriptomics and reverse vaccinology approaches. Despite the absence of *Mycoplasma pneumoniae* like cyto-adhesion encoding genes, the two strains showed the presence of protrusions. This phenotype is likely encoded by another set of genes encoding surface- and transmembrane proteins.

Mulungo, M., Frey, J., Smith, K., Schnier, C., Wesonga, H., Naessens, J. and McKeever, D. (2015) Vaccination of cattle with the LppQ-N′ sub-unit of *Mycoplasma mycoides* subsp. *mycoides* results in type III immune complex disease upon experimental infection. Infect. Immun. **83**:1992-2000

Fischer A., Santana-Cruz I., Hegerman J., Gourlé H., Schieck E., Lambert M., Nadendla S., Wesonga H., Miller R.A., Vashee S., Weber J., Meens J., Frey J, and Jores J. (2015) High quality draft genomes of the *Mycoplasma mycoides* subsp. *mycoides* challenge strains Afadé and B237. Standards in Genomic Sciences **10**: 89.

### 2.1.3. Virulence, persistence and dissemination of *Mycoplasma bovis.*

Paola Pilo, Sibylle Bürki
In collaboration with Prof. Michael Stoffel, Division of Veterinary Anatomy, University of Bern

Bovine mycoplasmosis due to *Mycoplasma bovis* causes several important bovine diseases such as pneumonia, mastitis, arthritis, otitis, genital disorders or keratoconjunctivitis. Variable surface lipoproteins, adhesion, invasion of host cells, modulation of the host immune system, biofilm formation and the release of secondary metabolites, as well as synergistic infections with other bacterial or viral pathogens are among the more significantly studied characteristics of the bacterium. A particularity of *M. bovis* infections is the broad range of clinical manifestations in cattle. They lead to enormous economic losses to the beef and dairy industries.

Antibiotic treatments are not efficacious and currently no efficient vaccine is available. Moreover, mechanisms of pathogenicity of this bacterium are not clear, as few virulence attributes are known. Microscopic observations of necropsy material suggest the possibility of an intracellular stage of *M. bovis.* We used a combination of a gentamicin protection assay, a variety of chemical treatments to block mycoplasmas entry in eukaryotic cells, and fluorescence and transmission electron microscopy to investigate the intracellular life of *M. bovis* in calf turbinate cells. Our findings indicate that *M. bovis* invades and persists in primary embryonic calf turbinate cells. Moreover, *M. bovis* can multiply within these cells. The intracellular phase of *M. bovis* may represent a protective niche for this pathogen and contribute to its escape from the host's immune defense as well as avoidance of antibiotic therapies.

Bürki, S., Frey, J. and Pilo, P. (2015) Virulence, persistence and dissemination of *Mycoplasma bovis.* Vet. Microbiol. **179**: 15–22.

Bürki S., Gaschen V., Stoffel M.H., Stojiljkovic A., Frey J., Kuehni-Goghenbor K. and Pilo P. (2015) Invasion and persistence of *Mycoplasma bovis* in embryonic calf turbinate cells. *Veterinary Research*, **46**:53

2.1.4. Identification of virulence attributes specific to ruminant rhombencephalitis in *Listeria monocytogenes* using comparative genomics

Collaborative Swiss National Science Foundation Sinergia project with Prof. Anna Oevermann, Division of Neurological Sciences, Department of Clinical Veterinary Medicine, University of Bern and Prof. Marc Lecuit, Institut Pasteur, Paris, France.

*Listeria monocytogenes* (LM) affects both ruminants and humans causing abortion, septicaemia, gastroenteritis and Central Nervous System (CNS) infections. Meningitis and meningoencephalitis are the most common central nervous system infections in humans, while rhombencephalitis is the main clinical form in cattle and small ruminants. In contrast to the human strains, LM in ruminants has been less studied and the molecular basics related to the invasion of CNS are not well known. LM strains are grouped in two main lineages I and II. Lineage I is composed mainly by clinical isolates from humans and ruminants. Lineage II clusters mostly environmental and food strains and some isolates from CNS diseases of small ruminants. The extensively investigated LM strain EGD-e belongs to lineage II. Lineage I strains have only scarcely been investigated although they constitute the virulent group. A goal in this first part of the project was to establish high quality genome sequences including their annotations from a characteristic strain of each LM lineage.

Whole genome alignment between JF5203 and F2365 reveals 99.99% of identity. The F2365 strain belongs to the same serovar 4b and was the responsible of an important listeriosis outbreak in California (2). On the other hand, JF4839 and the very well studied EGDe strain shared 99.92% of identity. These results confirm that LM strains of the same lineage are highly similar. However, a genome wide comparison of the rhombencephalitis strain JF5203 and the food strain JF4839 shows 5.7% differences. These regions are then our further target, to investigate potential genes or regulatory elements that facilitate the invasion of the bacteria producing CNS disorders.

As a result of the annotation step a set of 2935 CDS out of 3117 genes, 68 tRNA, 18 rRNA, 96 ncRNA, 1 CRISPRs and 27 internalin genes for JF5203 was predicted;

while for JF4839 we could identify 3013 CDS out of 3199 genes, 68 tRNA, 18 rRNA, 100 ncRNA, 1 CRISPRs and 28 internalin genes. Internalins together with LIPI-1 island are the main virulence elements.

In a molecular epidemiological study, *L. monocytogenes* was isolated from a lamb with septicemia and from the brainstem of three sheep with encephalitis. Samples from the farm environment (animal faeces, feeds including silage, soil, swabs from feed bunk, fodder mixing vehicle, water tank and floor) were screened for the presence of *L. monocytogenes* during the listeriosis outbreak, four weeks and eight months after. *L. monocytogenes* were found only in soil and water tank swabs during the outbreak. Four weeks later, following thorough cleaning of the barn, as well as eight months later, *L. monocytogenes* was absent in environmental samples. All environmental and clinical *L. monocytogenes* isolates were found to be the same strain. Our results indicate that the outbreak involving two different clinical syndromes was caused by a single *L. monocytogenes* strain and that soil and water tanks were potential infection sources during this outbreak. However, silage cannot be completely ruled out as the bales fed prior to the outbreak were not available for analysis. Faeces samples were negative, suggesting that sheep did not act as amplification hosts contributing to environmental contamination. In conclusion, farm management appears to be a crucial factor for the limitation of a listeriosis outbreak

Central nervous system (CNS) infections in ruminant livestock, such as listeriosis, are of major concern for veterinary and public health. To date, no pertinent host specific in vitro models for ruminant CNS infections are available. Here, we evaluated the suitability of organotypic brain slices of ruminant origin as in vitro model to study mechanisms of *L. monocytogenes* infection. Brains were obtained from young ruminants at the slaughterhouse and hippocampal and cerebellar brain-slices were cultured up to 49 days. Viability as well as composition of cell populations was assessed weekly. Viable neurons, astrocytes, microglia and oligodendrocytes were observed up to 49 days in vitro. Slice cultures were infected with *L. monocytogenes*, and infection kinetics were monitored. Infected brain cells were identified by double-immunofluorescence and results were compared to natural cases of listeric rhombencephalitis. Similar to the natural infection, infected brain slices showed focal replication of *L. monocytogenes* and bacteria were predominantly observed in microglia, but also in astrocytes, and associated with axons. Taken together, we show that organotypic brain-slice cultures can serve as a host-specific *in vitro* model to investigate host-pathogen interactions in listeriosis. In the future, organotypic brain-slice cultures of ruminant origin could also support research on other, even yet unknown, neuroinfectious diseases.

Dreyer, M., Thomann, A., Böttcher, S., Frey, J. and Oevermann, A. (2015) Outbreak investigation identifies a single *Listeria monocytogenes* strain in sheep with different clinical manifestations, soil and water. Vet. Microbiol. **179**:69–75.

Guldimann C., Bärtschi M., Frey J., Zurbriggen A., Torsten Seuberlich T. and Oevermann A. (2015) Increased spread and replication efficiency of *Listeria monocytogenes* in organotypic brain-slices is related to multilocus variable number of tandem repeat analysis (MLVA) complex. BMC Microbiology **15**:134.

Henke D., Rupp S., Gaschen V., Stoffel M.H., Frey J., Vandevelde M., Oevermann A. (2015) *Listeria monocytogenes* spreads within the brain by actin-based intra-axonal migration. Infection and Immunity **83**: 2409-2419.

Rupp, S., Aguilar-Bultet, L., Jagannathan, V., Guldimann, C., Drögemüller, C., Pfarrer, C., Bicono, B., Seuberlich, T., Frey, J. and Oevermann, A. (2015) A naturally occurring *prfA* truncation in a *Listeria monocytogenes* field strain contributes to reduced replication and cell-to-cell spread Vet. Microbiol. **179:** 91-101

### 2.1.5. Ecology of *Francisella tularensis*

*Francisella tularensis* is a zoonotic bacterium emerging in Europe. This microorganism is the causative agent of tularemia. This disease mainly affects rodents and lagomorphs but the biological cycle of the bacterium is poorly understood. We are using an interdisciplinary approach to investigate the molecular epidemiology, pathobiology, persistence and spread of *F. tularensis* in the environment *sensu lato*. To achieve this goal we are using several disciplines such as classical bacteriology, molecular biology, pathology and mathematical modelling.

Dobay A., Pilo P., Lindholm A.K., Origgi F., Bagheri H.C., König B. (2015) Dynamics of a Tularemia Outbreak in a Closely Monitored Free-Roaming Population of Wild House Mice. PLoS One. **10**(11):e0141103.
Doi: 10.1371/journal.pone.0141103

Ernst M., Pilo P., Fleisch F., Glisenti P. (2015) Tularemia in the Southeastern Swiss Alps at 1,700 m above sea level. Infection. **43**(1):111-5.
Doi: 10.1007/s15010-014-0676-3.

Longo M.V., Jaton K., Pilo P., Chabanel D., Erard V. (2015) Long-Lasting Fever and Lymphadenitis: Think about *F. tularensis*. Case Rep Med. 2015:191406.
Doi: 10.1155/2015/191406.

Origgi F.C., König B., Lindholm A.K., Mayor D., Pilo P. (2015) Tularemia among free-ranging mice without infection of exposed humans, Switzerland, 2012. Emerg Infect Dis. **21**(1):133-5.
Doi: 10.3201/eid2101.140906.

### 2.1.6. Source attribution of human *Campylobacter* infections

Peter Kuhnert and Romie Jonas

*Campylobacter jejuni* and *Campylobacter coli* are the most important bacterial causes of human gastroenteritis. Chicken has been recognized as a major source for human infection, whereas cattle might also contribute to a lesser extent. However, there is a paucity of information available regarding *Campylobacter* in Swiss cattle and their role for human campylobacteriosis. To gain more information on genotypes and antibiotic resistance of bovine *C. jejuni* and *C. coli* and on their contribution to human disease, 97 cattle isolates were analysed. Multilocus sequence typing (MLST) and *flaB* typing were applied and the *gyrA* and 23S rRNA genes were screened for point mutations responsible for quinolone and macrolide resistance, respectively. A total of 37 sequence types (STs) and 44 *flaB* types were identified, including two

sequence types and five *flaB* types not previously described. Most common sequence types were ST21 (21%), ST61 (12%) and ST48 (11%). Only one isolate was macrolide resistant while 31% (n = 30) were quinolone resistant. Source attribution indicated chicken as the main source of human infection with cattle being second. In conclusion, cattle should not be underestimated as a potential source of human campylobacteriosis.

Jonas R., Kittl S., Overesch G., Kuhnert P. (2015) Genotypes and antibiotic resistance of bovine Campylobacter and their contribution to human campylobacteriosis. Epidemiol Infect. **143**:2373-2380.
Doi: 10.1017/S0950268814003410.

## 2.1.7. *Uruburuella testudinis* sp. nov., isolated from tortoise (*Testudo*)

Peter Kuhnert

A polyphasic taxonomic analysis was carried out on 11 uncommon Gram-stain-negative, non-motile, catalase- and oxidase-positive, but indole-negative, bacterial strains isolated from tortoises. Phenotypically and genetically they represented a homogeneous group of organisms most closely related to, but distinct from, *Uruburuella suis*. In a reconstructed 16S rRNA gene tree they clustered on a monophyletic branch next to *U. suis* with gene similarities between strains of 99.5-100 %, and of up to 98.2 % with *U. suis*. DNA-DNA hybridization indicated the organisms represented a novel species with only 40 % DNA-DNA similarity with U. suis. Partial sequencing of *rpoB* resulted in two subclusters confirming the 16S rRNA gene phylogeny; both genes allowed clear separation and identification of the novel species. Furthermore, they could be unambiguously identified by matrix-assisted laser desorption ionization time-of-flight MS, where, again, they formed a highly homogeneous cluster separate from *U. suis* and other members of the family *Neisseriaceae*. The major fatty acids were C16 : 0 and summed feature C16 : 1ϖ7c/iso-C15 : 0 2-OH. The DNA G+C content was 54.4 mol%. Based on phenotypic and genetic data we propose classifying these organisms as representatives of a novel species named *Uruburuella testudinis* sp. nov. The type strain is 07_OD624$^T$ (=DSM 26510$^T$=CCUG 63373$^T$).

Kuhnert P., Thomann A., Brodard I., Haefeli W., Korczak B.M. (2015) *Uruburuella testudinis* sp. nov., isolated from tortoise (*Testudo*). Int J Syst Evol Microbiol **65**:1251-1255.
Doi: 10.1099/ijs.0.000089

## 2.1.8. Reclassification of *Actinobacillus muris* as *Muribacter muris* gen. nov., comb. nov.

Peter Kuhnert, in collaboration with Magne Bisgaard and Henrik Christensen, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen

To reinvestigate the taxonomy of [*Actinobacillus*] *muris*, 474 strains, mainly from mice and rats, were characterized by phenotype and 130 strains selected for genotypic characterization by 16S rRNA and partial *rpoB* gene sequencing. The type strain was

further investigated by whole-genome sequencing. Phylogenetic analysis of the DNA sequences showed one monophyletic group with intragroup similarities of 96.7 and 97.2 % for the 16S rRNA and *rpoB* genes, respectively. The highest 16S rRNA gene sequence similarity to a taxon with a validly published name outside the group was 95.9 %, to the type strain of [*Pasteurella*] *pneumotropica*. The closest related taxon based on *rpoB* sequence comparison was "*Haemophilus influenzae-murium*", with 88.4 % similarity. A new genus and a new combination, *Muribacter muris* gen. nov., comb. nov., are proposed based on a distinct phylogenetic position based on 16S rRNA and *rpoB* gene sequence comparisons, with major divergence from the existing genera of the family *Pasteurellaceae*. The new genus has the characteristics of [*A.*] *muris* with the emendation that acid formation from (-)-D-mannitol and hydrolysis of aesculin are variable, while the $\alpha$-glucosidase test is positive. There is no requirement for exogenously supplied NAD (V factor) for the majority of strains investigated; however, one strain was found to require NAD. The major fatty acids of the type strain of *Muribacter muris* were C14 : 0,C14 : 0 3-OH/iso-C16 : 1 I, C16 : 1$\omega$7c and C16 : 0, which is in line with most genera of the *Pasteurellaceae*. The type strain of *Muribacter muris* is CCUG 16938$^T$ (=NCTC 12432$^T$=ATCC 49577$^T$).

Nicklas W., Bisgaard M., Aalbæk B., Kuhnert P., Christensen H. Reclassification of *Actinobacillus muris* as *Muribacter muris* gen. nov., comb. nov. Int J Syst Evol Microbiol 65:3344-3351.
Doi: 10.1099/ijsem.0.000417.

## 2.2. Molecular and Bacterial Epidemiology and Infectiology

**Vincent Perreten**

### 2.2.1. A novel universal DNA labelling and amplification system for rapid microarray-based detection of 117 antibiotic resistance genes in Gram-positive bacteria.

A rapid and simple DNA labeling system has been developed for disposable microarrays and has been validated for the detection of 117 antibiotic resistance genes abundant in Gram-positive bacteria. The DNA was fragmented and amplified using phi-29 polymerase and random primers with linkers. Labeling and further amplification were then performed by classic PCR amplification using biotinylated primers specific for the linkers. The microarray developed by Perreten et al. (Perreten, V., Vorlet-Fawer, L., Slickers, P., Ehricht, R., Kuhnert, P., Frey, J., 2005. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria J.Clin.Microbiol. 43, 2291-2302.) was improved by additional oligonucleotides. A total of 244 oligonucleotides (26 to 37 nucleotide length and with similar melting temperatures) were spotted on the microarray, including genes conferring resistance to clinically important antibiotic classes like β-lactams, macrolides, aminoglycosides, glycopeptides and tetracyclines. Each antibiotic resistance gene is represented by at least 2 oligonucleotides designed from consensus sequences of gene families. The specificity of the oligonucleotides and the quality of the amplification and labeling were verified by analysis of a collection of 65 strains belonging to 24 species. Association between genotype and phenotype was verified for 6 antibiotics using 77 *Staphylococcus* strains belonging to different species and revealed 95% test specificity and a 93% predictive value of a positive test. The DNA labeling and amplification is independent of the species and of the target genes and could be used for different types of microarrays. This system has also the advantage to detect several genes within one bacterium at once, like in *Staphylococcus aureus* strain BM3318, in which up to 15 genes were detected. This new microarray-based detection system offers a large potential for applications in clinical diagnostic, basic research, food safety and surveillance programs for antimicrobial resistance.

Publication: Strauss C, Endimiani A, Perreten V. (2015) J. Microbiol. Methods. 108:25-30.

### 2.2.2. First report of a multidrug-resistant *Klebsiella pneumoniae* of sequence type 11 causing sepsis in a free-ranging beaver (*Castor fiber*).

*Klebsiella pneumoniae* of sequence type (ST) 11 is a hyper-epidemic nosocomial clone spreading worldwide among humans and also emerging in pets. In this report, we describe a clinical case of fatal sepsis due to this multidrug-resistant (MDR) pathogen in a Eurasian beaver. The isolate showed resistance to six different classes of antimicrobials including third generation cephalosporins and fluoroquinolones. This is the first report describing the detection of a MDR *K. pneumoniae* ST11 in a free-ranging animal. Our finding highlights the potential for environmental dissemination of

hyper-epidemic clones of *K. pneumoniae* and possible spread in wildlife and cause epizootics.

Publication: Pilo P., Vogt D., Origgi F.C., Endimiani A., Peterson S., Perreten V. (2015) Environ Microbiol Rep. **7**(2):351-3.

### 2.2.3. Third-generation-cephalosporin-resistant *Klebsiella pneumoniae* isolates from humans and companion animals in Switzerland: spread of a DHA-producing sequence type 11 clone in a veterinary setting

Characterization of third-generation-cephalosporin-resistant *Klebsiella pneumoniae* isolates originating mainly from one human hospital (n = 22) and one companion animal hospital (n = 25) in Bern (Switzerland) revealed the absence of epidemiological links between human and animal isolates. Human infections were not associated with the spread of any specific clone, while the majority of animal infections were due to *K. pneumoniae* sequence type 11 isolates producing plasmidic DHA AmpC. This clonal dissemination within the veterinary hospital emphasizes the need for effective infection control practices.

Publication: Wohlwend N., Endimiani A., Francey T., Perreten V. (2015). Antimicrob Agents Chemother. **59**(5):2949-55.

### 2.2.4. New shuttle vector-based expression system to generate polyhistidine-tagged fusion proteins in *Staphylococcus aureus* and *Escherichia coli*.

Four *Staphylococcus aureus-Escherichia coli* shuttle vectors were constructed for gene expression and production of tagged fusion protein. Vectors pBUS1-HC and pTSSCm are promoter-less for studying gene under control of their native promoter, and pBUS1-$P_{cap}$-HC and pTSSCm-$P_{cap}$ contain the strong constitutive promoter of *S. aureus* type 1 capsule gene 1A ($P_{cap}$) upstream of a novel multiple cloning site (MCS) harboring codons for the peptide tag Arg-Gly-Ser-hexa-His (*rgs-his*$_6$). All plasmids contained the backbone derived from pBUS1 including the *E. coli* origin ColE1, the terminator *rrnB*$(T1)_5$ and the tetracycline resistance marker *tet*(L) for *S. aureus* and *E. coli*. Gram-positive replicon was improved through either complementation of the minimum pAMα1 replicon from pBUS1 with the single strand origin *oriL* from pUB110 (pBUS1-HC and pBUS1-$P_{cap}$-HC) or substitution with a pT181-family replicon (pTSSCm and pTSSCm-$P_{cap}$). The new constructs displayed increased plasmid yield and segregational stability in *S. aureus*. Furthermore, pBUS1-$P_{cap}$-HC and pTSSCm-$P_{cap}$ offer the potential to generate C-terminal RGS-$His_6$ translational fusions of cloned genes using simple molecular manipulation. BcgI-induced DNA excision followed by religation converts the TGA stop codon of the MCS into a TGC codon and links the *rgs-his*$_6$ codons to the 3' end of the target gene. The generation of the *rgs-his*$_6$ codon-fusion, gene expression and protein purification were demonstrated in both *S. aureus* and *E. coli* using the macrolide, lincosamide, streptogramin B resistance gene *erm*(44) inserted downstream of $P_{cap}$. The new His-tag expression

system represents a helpful tool for the direct analysis of target gene function in staphylococcal cells.

Publication: Schwendener S., Perreten V. (2015) Appl Environ Microbiol. **81**(9):3243-54.

2.2.5. The new macrolide-lincosamide-streptogramin B resistance gene *erm*(45) is located within a genomic island in *Staphylococcus fleurettii*.

Genome alignment of a macrolide, lincosamide, and streptogramin B ($MLS_B$)-resistant *Staphylococcus fleurettii* strain with an MLSB-susceptible *S. fleurettii* strain revealed a novel 11,513-bp genomic island carrying the new erythromycin resistance methylase gene *erm*(45). This gene was shown to confer inducible $MLS_B$ resistance when cloned into *Staphylococcus aureus*. The *erm*(45)-containing island was integrated into the housekeeping gene *guaA* in *S. fleurettii* and was able to form a circular intermediate but was not transmissible to *S. aureus*.

Publication: Wipf J.R., Schwendener S., Nielsen J.B., Westh H., Perreten V. (2015) Antimicrob Agents Chemother. **59**(6):3578-81.

2.2.6. First Staphylococcal Cassette Chromosome *mec* containing a *mecB*-carrying gene complex independent of transposon Tn*6045* in a *Macrococcus caseolyticus* isolate from a canine infection.

A methicillin-resistant *mecB*-positive *Macrococcus caseolyticus* (strain KM45013) was isolated from the nares of a dog with rhinitis. It contained a novel 39-kb transposon-defective complete *mecB*-carrying SCC*mec* element (SCC*mec*$_{KM45013}$). SCC*mec*$_{KM45013}$ contained 49 coding sequences (CDS), was integrated at the 3' end of the chromosomal *orfX*, and was delimited at both ends by imperfect direct repeats functioning as Integration Site Sequences (ISSs). SCC*mec*$_{KM45013}$ presented two discontinuous regions of homology (SCC*mec* coverage of 35%) to the chromosomal and transposon Tn*6045*-associated SCC*mec*-like element of *M. caseolyticus* JCSC7096: (i) the *mec* gene complex (98.8% identity) and (ii) the *ccr*-carrying segment (91.8%). The *mec* gene complex, located at the right junction of the cassette, also carried a β-lactamase gene *blaZm* (*mecRm-mecIm-mecB-blaZm*). SCC*mec*$_{KM45013}$ contained two cassette chromosome recombinase genes, *ccrAm2* and *ccrBm2* which shared 94.3 and 96.6% DNA identity with those of the SCC*mec*-like element of JCSC7096, but less than 51% DNA identity with the staphylococcal *ccrAB* and *ccrC* genes. Three distinct extrachromosomal circularized elements flanked by one ISS copy (the entire SCC*mec*$_{KM45013}$, ψSCC*mec*$_{KM45013}$ lacking the *ccr* genes, and SCC$_{KM45013}$ lacking *mecB*) as well as the chromosomal regions remaining after excision were detected. An uncommon circularized structure (UCS) carrying the *mecB* gene complex was associated with two extensive direct repeat regions, which enclosed two ORFs (ORF46 and ORF51) flanking the chromosomal *mecB*-carrying gene complex. This study revealed *M. caseolyticus* as a potential disease-associated

bacteria in dog and secondly, unveiled a SCC*mec* element carrying *mecB* not associated with Tn*6045* in the genus *Macrococcus*.

Publication: Gómez-Sanz E., Schwendener S., Thomann A., Gobeli Brawand S., Perreten V. (2015) Antimicrob Agents Chemother. **59**(8):4577-83.

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## 3. ZOBA – Centre for Zoonoses, Bacterial Animal Diseases and Antimicrobial Resistance

**Gudrun Overesch**

In the division diagnostic and surveillance a total of 15165 samples, resp. animals were analysed. 11 analyses were conducted for the veterinary border control. Regarding the antimicrobial resistance monitoring program 4294 isolations for various pathogens, multi-resistant bacteria and indicator microorganisms were performed. Moreover 1138 analyses using the Minimal Inhibitory Concentration technique (MIC) were conducted. Furthermore, on behalf of the reference function for a broad variety of epizootics, our laboratory carried out 434 confirmations. Further details are shown in the tables below.

**Diagnostic activities**

| Division | Number of samples/animals |
|---|---|
| Clinical material and mycology | 2570 animals |
| Necropsy material, abortion and feaces | 1872 animals |
| Surveillance | 1849 animals |
| Bovine mastitis | 3061 samples |
| Serology | 2890 animals |
| Identification and molecular diagnostics | 1480 samples |
| Antibiograms for diagnostics | 1443 analyses |
| Antimicrobial resistance monitoring (detection) | 4294 analyses |
| Antimicrobial resistance monitoring (MIC*) | 1138 analyses |
| Veterinary border control | 11 analyses |
| Reference function | 434 samples |

* Minimal inhibitory concentration

### 3.1. Diagnostic Activity for Epizootics (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 |

### 3.1.1. Highly infectious diseases

| Epizootic | Agent | Method | Animal | Total | negative | suspi-cious | positive |
|---|---|---|---|---|---|---|---|
| **Contagious bovine pleuropneumonia** | *Mycoplasma mycoides* subsp. *mycoides* | Culture | **cattle** | 0 | 0 | 0 | 0 |
| | | ELISA | | 0 | 0 | 0 | 0 |
| | | Immuno-blot | | 0 | 0 | 0 | 0 |

### 3.1.2. Diseases to be eradicated

| Epizootic | Agent | Method | Animal | Total | nega-tive | suspi-cious | posi-tive |
|---|---|---|---|---|---|---|---|
| **Anthrax** | *Bacillus anthracis* | Micr | **cattle** | 4 | 4 | 0 | 0 |
| | | Cult | | 4 | 4 | 0 | 0 |
| | *Bacillus anthracis* | Micr | **cat** | 1 | 1 | 0 | 0 |
| | | Cult | | 1 | 1 | 0 | 0 |
| | *Bacillus anthracis* | Micr | **swine** | 0 | 0 | 0 | 0 |
| | | Cult | | 0 | 0 | 0 | 0 |
| **Brucellosis** | *Brucella abortus* | Micr | **cattle** | 318 | 308 | 10 | 0 |
| | | RBT | | 0 | 0 | 0 | 0 |
| | | ELISA | | 711 | 707 | 1 | 3 |
| | | CFT | | 5 | 5 | 0 | 0 |
| | *Brucella melitensis* | Micr | **sheep/goat** | 57 | 53 | 4 | 0 |
| | | ELISA | | 26 | 26 | 0 | 0 |
| | | CFT | | 0 | 0 | 0 | 0 |
| | | RBT | | 0 | 0 | 0 | 0 |
| | *Brucella abortus / Brucella melitensis* | Micr | **diverse** | 21 | 21 | 0 | 0 |
| | | ELISA | | 46 | 46 | 0 | 0 |
| | | CFT | | 3 | 3 | 0 | 0 |
| | | RBT | **diverse** | 9 | 9 | 0 | 0 |
| | | | **alpaca** | 23 | 23 | 0 | 0 |
| | | | **deer** | 5 | 5 | 0 | 0 |
| | | | **ibex/ chamois** | 7 | 7 | 0 | 0 |
| | *Brucella suis* | Micr | **swine** | 27 | 27 | 0 | 0 |
| | | RBT | | 721 | 719 | 1 | 1 |
| | | CFT | | 2 | 0 | 2 | 0 |
| | *Brucella ovis* | ELISA | **sheep** | 22 | 19 | 2 | 1 |
| | | | **others** | 0 | 0 | 0 | 0 |
| | *Brucella canis* | Micr | **dog** | 0 | 0 | 0 | 0 |
| | | LF | | 5 | 5 | 0 | 0 |

| Epizootic | Agent | Method | Animal | Total | negative | suspici-ous | positive |
|---|---|---|---|---|---|---|---|
| **Bovine Campylo-bacteriosis** | *Campylobacter fetus* subspecies *venerealis* | Cult | **cattle** | 841 | 742 | 99 | 0 |
| | | PCR | | 99 | 99 | 0 | 0 |
| **Infectious agalactia** | *Mycoplasma agalactiae* | ELISA | **goat** | 0 | 0 | 0 | 0 |

### 3.1.3. Diseases to be controlled

| Epizootic | Agent | Method | Animal | Total | negative | suspi-cious | positive |
|---|---|---|---|---|---|---|---|
| **Leptospirosis** | *L.* Australis | MAT | **cattle** | 449 | 449 | 0 | 0 |
| | | | **dog** | 116 | 74 | 18 | 24 |
| | | | **horse** | 16 | 9 | 4 | 3 |
| | | | **swine** | 5 | 5 | 0 | 0 |
| | *L.* Autumnalis | MAT | **dog** | 115 | 98 | 11 | 6 |
| | | | **horse** | 16 | 13 | 2 | 1 |
| | *L.* Ballum | MAT | **dog** | 7 | 7 | 0 | 0 |
| | | | **swine** | 5 | 5 | 0 | 0 |
| | *L.* Bataviae | MAT | **dog** | 113 | 111 | 2 | 0 |
| | | | **horse** | 9 | 9 | 0 | 0 |
| | *L.* Bratislava | MAT | **cattle** | 7 | 7 | 0 | 0 |
| | | | **dog** | 115 | 82 | 11 | 22 |
| | | | **horse** | 16 | 10 | 2 | 4 |
| | | | **swine** | 19 | 19 | 0 | 0 |
| | *L.* Canicola | MAT | **cattle** | 456 | 456 | 0 | 0 |
| | | | **dog** | 114 | 98 | 14 | 2 |
| | | | **horse** | 16 | 14 | 2 | 0 |
| | | | **swine** | 5 | 5 | 0 | 0 |
| | *L.* Copenhageni | MAT | **dog** | 96 | 70 | 19 | 7 |
| | *L.* Grippotyphosa | MAT | **cattle** | 456 | 455 | 1 | 0 |
| | | | **dog** | 117 | 103 | 9 | 5 |
| | | | **horse** | 16 | 12 | 4 | 0 |
| | | | **swine** | 5 | 5 | 0 | 0 |
| | *L.* Hardjo | MAT | **cattle** | 560 | 514 | 37 | 9 |
| | | | **dog** | 112 | 111 | 1 | 0 |
| | | | **horse** | 9 | 9 | 0 | 0 |
| | *L.* Icterohaemo-rrhagiae | MAT | **cattle** | 456 | 456 | 0 | 0 |
| | | | **dog** | 117 | 113 | 4 | 0 |
| | | | **horse** | 16 | 14 | 2 | 0 |
| | | | **swine** | 19 | 19 | 0 | 0 |

| Epizootic | Agent | Method | Animal | Total | negative | suspi-cious | positive |
|---|---|---|---|---|---|---|---|
| **Leptospirosis** | *L.* Pomona | MAT | **cattle** | 456 | 456 | 0 | 0 |
| | | | **dog** | 115 | 95 | 15 | 5 |
| | | | **horse** | 16 | 12 | 4 | 0 |
| | | | **swine** | 19 | 19 | 0 | 0 |
| | *L.* Pyrogenes | MAT | **dog** | 106 | 104 | 2 | 0 |
| | | | **horse** | 14 | 11 | 3 | 0 |
| | *L.* Sejroe | MAT | **cattle** | 457 | 444 | 12 | 1 |
| | | | **dog** | 30 | 30 | 0 | 0 |
| | | | **horse** | 9 | 8 | 1 | 0 |
| | *L.* Tarasosovi | MAT | **cattle** | 457 | 454 | 3 | 0 |
| | | | **dog** | 113 | 111 | 2 | 0 |
| | | | **horse** | 16 | 15 | 1 | 0 |
| | | | **swine** | 19 | 19 | 0 | 0 |
| | *Leptospira* spp | MAT eye | **horse** | 1 | 1 | 0 | 0 |
| | | PCR | **diverse** | 44 | 42 | 0 | 2 |

| Epizootic | Agent | Animal | Total | negative | suspi-cious | posi-tive |
|---|---|---|---|---|---|---|
| **Salmonellosis** | | **alpaca** | 2 | 2 | 0 | 0 |
| (Cult) | | **antilope** | 1 | 1 | 0 | 0 |
| | | **ape** | 26 | 26 | 0 | 0 |
| | | **bear** | 2 | 2 | 0 | 0 |
| | | **bird** | 37 | 36 | 0 | 1 |
| | *S.* Enteritidis | | | | | 1 |
| | | **cat** | 21 | 21 | 0 | 0 |
| | | **cattle** | 278 | 243 | 0 | 35 |
| | *S.* Typhimurium | | | | | 2 |
| | *S.* Stockholm | | | | | 32 |
| | *S.* Dublin | | | | | 1 |
| | *S.* Typhimurium, monophasic variant (4,12 : i : -) | | | | | 3 |
| | | **chicken** | 13 | 13 | 0 | 0 |
| | | **deer** | 2 | 2 | 0 | 0 |
| | | **dog** | 53 | 50 | 0 | 3 |
| | *S.* Indiana | | | | | 2 |
| | *S. enterica* subsp. *enterica* 9,12:l,v:- | | | | | 1 |
| | | **donkey** | 4 | 4 | 0 | 0 |
| | | **duck** | 6 | 6 | 0 | 0 |
| | | **elephant** | 4 | 4 | 0 | 0 |

| Epizootic | Agent | Animal | Total | negative | suspi-cious | posi-tive |
|---|---|---|---|---|---|---|
| **Salmonellosis** | | **goat** | 28 | 28 | 0 | 0 |
| (Cult) | | **hedgehoge** | 1 | 1 | 0 | 0 |
| | | **horse** | 60 | 60 | 0 | 0 |
| | | **iguana** | 4 | 2 | 0 | 2 |
| | *S. enterica* subsp. *houtenae* 45:g,z51:- | | | | | 1 |
| | *S. enterica* subsp. *houtenae* 50:g,z51:- | | | | | 1 |
| | | **parrot** | 6 | 6 | 0 | 0 |
| | | **saurian** | 5 | 3 | 0 | 2 |
| | *S. enterica* subsp. *houtenae* 44:z4,z24:- | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 50:k:z | | | | | 1 |
| | | **sheep** | 53 | 42 | 0 | 11 |
| | *S. enterica* subsp. *diarizonae* 61:k:1,5,7 | | | | | 11 |
| | | **snake** | 24 | 10 | 0 | 14 |
| | *S. enterica* subsp. *arizonae* 41:z4,z23,-* | | | | | 1 |
| | *S. enterica* subsp. *arizonae* rough z4,z23:-* | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 11:l,v:z | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 16:z10:e,n,x,z15 | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 35:k:e,n,x,z15 | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 38:k:-* | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 50:k:- | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* rough l,v:1,5 | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 47:k:z35* | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 61:k:z35* | | | | | 2 |
| | *S. enterica* subsp. *diarizonae* 47:z52:e,n,x,y,z15* | | | | | 1 |
| | *S. enterica* subsp. *diarizonae* 48:l,v:1,5,7* | | | | | 1 |
| | *S.* Paratyphi B | | | | | 1 |
| | | **swine** | 111 | 110 | 0 | 1 |
| | *S.* Ohio | | | | | 1 |
| | | **tortoise** | 6 | 6 | 0 | 0 |
| | | **wild animal** | 4 | 4 | 0 | 0 |
| | | **zoo animal** | 32 | 32 | 0 | 0 |

* serotyping in human reference laboratory

| Epizootic | Agent | Method | Animal | Total | negative | suspi-cious | positive |
|---|---|---|---|---|---|---|---|
| **Contagious equine Metritis** | *Taylorella equigenitalis* | Cult | **horse** | 62 | 62 | 0 | 0 |
| **Enzootic pneumonia in swine** | *Mycoplasma hyopneumoniae* | PCR Lung | **swine** | 133 | 125 | 0 | 8 |
| | | PCR Nasal swab | | 41 | 35 | 5 | 1 |
| | | ELISA | | 152 | 145 | 1 | 6 |
| **Swine actinobacillosis** | *Actinobacillus pleuropneumoniae* | Cult | **swine** | | 83 | 72 | 11 |
| | | PCR | BvI I BD+II CA | | Serotyp 7,12 | | 6 |
| | | | BVI I CA+ BD | | Serotyp 10 | | 2 |
| | | | BVII I BD+II CA+III CA+BD | | Serotyp 2 | | 2 |
| | | | BV II | | not defined | | 1 |
| | | ELISA ApxIV | | 12 | 12 | 0 | 0 |

### 3.1.4. Diseases to be monitored

| Epizootic | Agent | Method | Animal | Total | nega-tive | suspi-cious | positive |
|---|---|---|---|---|---|---|---|
| **Paratuberculosis** | *Mycobacterium avium* subspecies *paratuberculosis* | Micr | **cattle** | 1 | 1 | 0 | 0 |
| **Campylo-bacteriosis** (thermotolerant) | | Cult | **dog** | 38 | 38 | 0 | 0 |
| | | | **cat** | 20 | 20 | 0 | 0 |
| | | | **monkey** | 16 | 16 | 0 | 0 |
| | | | **diverse** | 6 | 6 | 0 | 0 |
| **Listeriosis** | *Listeria monocytogenes* | Cult | **cattle** | 4 | 1 | 0 | 3 |
| | | Cult | **diverse** | 3 | 1 | 0 | 2 |
| **Yersiniosis** | *Yersinia* spp. | Cult | **dog** | 0 | 0 | 0 | 0 |
| | | Cult | **diverse** | 16 | 7 | 0 | 9 |
| **Caseous lymphadenitis in sheep and goats** | *Corynebacterium pseudotubercu-losis (ovis)* | Cult | **goat** | 6 | 5 | 0 | 1 |
| | | Cult | **sheep** | 2 | 1 | 0 | 1 |

| Epizootic | Agent | Method | Animal | Total | nega-tive | suspi-cious | positive |
|---|---|---|---|---|---|---|---|
| **Enzootic abortion in ewes (chlamy-diosis)** | *Chlamydophila abortus* | Micr | **sheep** | 19 | 17 | 2 | 0 |
| | | ELISA | | 27 | 13 | 0 | 14 |
| | | PCR | | 14 | 8 | 0 | 6 |
| | | Micr | **goat** | 31 | 30 | 1 | 0 |
| | | ELISA | | 30 | 19 | 0 | 11 |
| | | PCR | | 19 | 18 | 0 | 1 |
| | | Micr | **cattle** | 150 | 145 | 5 | 0 |
| | | ELISA | | 187 | 94 | 23 | 70 |
| | | PCR | | 133 | 133 | 0 | 0 |
| **Psittacosis** | *Chlamydophila psittaci* | PCR | **bird** | 1 | 1 | 0 | 0 |
| **Tularaemia** | *Francisella tularensis* | Cult | **monkey** | 2 | 2 | 0 | 0 |
| | | Cult | **beaver** | 3 | 2 | 1 | 0 |
| | | Cult | **hare** | 21 | 11 | 4 | 6 |
| **Blackleg** | *Clostridium chauvoei* | IF | **cattle** | 10 | 4 | 0 | 6 |
| | | Cult | | 10 | 8 | 0 | 2 |
| **Coxiellosis** | *Coxiella burnetii* | Micr | **cattle** | 162 | 157 | 5 | 0 |
| | | ELISA | | 122 | 110 | 0 | 12 |
| | | PCR | | 135 | 133 | 0 | 2 |
| | | Micr | **sheep** | 20 | 18 | 2 | 0 |
| | | ELISA | | 13 | 13 | 0 | 0 |
| | | PCR | | 14 | 14 | 0 | 0 |
| | | Micr | **goat** | 30 | 29 | 1 | 0 |
| | | ELISA | | 10 | 10 | 0 | 0 |
| | | PCR | | 18 | 18 | 0 | 0 |

## 3.2. Antimicrobial Resistance Monitoring

Programme concerning Swiss food producing animals

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. Caecal samples from pigs and calves were collected at slaughter and cultured for *E. coli*, *Enterococcus* spp., *Campylobacter* spp., Extended Spectrum beta-Lactamases (ESBLs) and Carbapenemases producing *E. coli*. Moreover fresh porcine and bovine meat from retails was analysed for ESBLs and Methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore nasal swabs from pigs and calves were tested for Methicillin-resistant *S. aureus* (MRSA). Isolated strains and all *S.* Typhimurium *and S.* Enteritidis strains from diagnostics and reference function were tested for antimicrobial susceptibility. For testing the Minimal Inhibitory Concentration (MIC) technique by broth microdilution was performed.

Results of the antimicrobial resistance monitoring are published nationalwide in the ARCH-Vet Report on the sales of antibiotics for veterinary use and antibiotic resistance monitoring of livestock in Switzerland 2015, Federal Food Safety and Veterinary Office (FSVO) and Swiss Agency for therapeutic products (Swissmedic). O nthe European level the results are published also in European Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015, European Food Safety Authority and European Centre for Disease Prevention and Control.

Number of analyses on *S. aureus* (MRSA)

| **Matrix** | **number of analyses** |
|---|---|
| pigs (nasal swabs) | 300 |
| Fresh porcine meat | 306 |
| Calves (nasal swabs) | 291 |
| Fresh bovine meat | 296 |
| **Total** | **1193** |

Number of analyses on ESBL/pAmpC producing *E. coli*

| **Matrix** | **number of analyses** |
|---|---|
| pigs (caecal samples) | 299 |
| Fresh porcine meat | 306 |
| Calves (caecal samples) | 299 |
| Fresh bovine meat | 296 |
| **Total** | **1200** |

Number of analyses on Carbapenemases producing *E. coli*

| **Matrix** | **number of analyses** |
|---|---|
| Fresh porcine meat | 306 |
| Fresh bovine meat | 296 |
| **Total** | **602** |

Number of analyses on *E. coli*

| **Matrix** | **number of analyses** |
|---|---|
| pigs (caecal samples) | 206 |
| Calves (caecal samples) | 196 |
| **Total** | **402** |

Number of analyses on *Enterococcus* spp.

| **Animal** | **number of analyses** |
|---|---|
| pigs (caecal samples) | 299 |
| Calves (caecal samples) | 299 |
| **Total** | **598** |

Number of analyses on *Campylobacter* spp.

| **Matrix** | **number of analyses** |
|---|---|
| pigs (caecal samples) | 299 |
| **Total** | **299** |

## 3.3. Reference Activity for Epizootics (Notifiable Animal Diseases)

### 3.3.1. Confirmation of results for other laboratories

| Epizootic | Method | Animal | Total | Negative | Suspicious | Positive |
|---|---|---|---|---|---|---|
| **Anthrax** | Micr | **Cattle** | 1 | 1 | 0 | 0 |
| | Cult | **Cattle** | 1 | 1 | 0 | 0 |
| **Bovine Brucellosis** | ELISA | **Cattle** | 9 | 2 | 4 | 3 |
| | RBT | **Cattle** | 9 | 6 | 0 | 3 |
| | CFT | **Cattle** | 9 | 8 | 0 | 1 |
| **Caprine and ovine Brucellosis** | ELISA | **Sheep/ goat** | 1 | 0 | 0 | 1 |
| | RBT | **Sheep/ goat** | 1 | 1 | 0 | 0 |
| | CFT | **Sheep/ goat** | 2 | 2 | 0 | 0 |
| **Porcine Brucellosis** | RBT | **Swine** | 0 | 0 | 0 | 0 |
| | CFT | **Swine** | 0 | 0 | 0 | 0 |
| **Canine Brucellosis** | LF | **Dog** | 1 | 1 | 0 | 0 |
| | Micr | **Dog** | 1 | 1 | 0 | 0 |
| | Cult | **Dog** | 1 | 1 | 0 | 0 |
| | Direct PCR | **Dog** | 1 | 1 | 0 | 0 |
| **Ovine epididymitis (*Brucella ovis*)** | ELISA | **Sheep** | 1 | 0 | 0 | 1 |
| **Swine actinobacillosis** | ELISA ApxIV | **Swine** | 0 | 0 | 0 | 0 |
| | Cult | **Swine** | 47 | 2 | 0 | 45 |
| | PCR | **Swine** | 45 | 0 | 0 | 45 |
| **Contagious equine Metritis** | Cult | **Horse** | 0 | 0 | 0 | 0 |
| | PCR | **Horse** | 0 | 0 | 0 | 0 |
| **Blackleg** | IF | **Cattle** | 4 | 3 | 0 | 1 |
| | Cult | **Cattle** | 3 | 2 | 0 | 1 |
| | PCR | **Cattle** | 0 | 0 | 0 | 0 |
| **Campylobacteriosis** | ID | **Dog** | 47 | 5 | 0 | 42 |
| | | **Cat** | 6 | 1 | 0 | 5 |
| | | **Chicken** | 2 | 0 | 0 | 2 |

| Epizootic | Method | Animal | Total | Nega-tive | Suspi-cious | Positive |
|---|---|---|---|---|---|---|
| **Campylobacteriosis** | ID | **Cattle** | 9 | 1 | 0 | 8 |
| | | **Sheep** | 1 | 0 | 0 | 1 |
| | | **Diverse** | 4 | 0 | 0 | 4 |
| **Enzootic pneumonia in swine** | PCR Lung | **Swine** | 2 | 1 | 0 | 1 |
| **Yersiniosis** | ID | **Fish** | 5 | 0 | 0 | 5 |
| **Multidrug Resistance** | ID, MIC | **Diverse** | 45 | 0 | 0 | 45 |

### 3.3.2. Serotyping of *Salmonella* sp. from other laboratories

| Serovar | Animal | Number |
|---|---|---|
| *S*. Abortusovis | sheep | 1 |
| *S*. Abony | chicken | 1 |
| *S*. Agona | chicken | 2 |
| *S*. Albany | chicken | 1 |
| | turkey | 1 |
| *S*. Cotham* | Saurian | 1 |
| *S*. Chester | chicken | 2 |
| | unknown | 2 |
| *S*. Dublin | cattle | 3 |
| *S.* Eboko | chicken | 2 |
| *S*. Enteritidis | cattle | 5 |
| | chicken | 11 |
| | leopard | 1 |
| | saurian | 1 |
| | owl | 1 |
| *S. enterica* subsp. *arizonae* 44: z4,z23: - | snake | 1 |
| *S. enterica* subsp. *arizonae* 48: z4,z23: - * | snake | 1 |
| *S*. *enterica* subsp. *diarizonae* 61: k: 1,5,7 | sheep | 2 |
| *S*. *enterica* subsp. *diarizonae* 42: z10:z67 * | snake | 1 |
| *S*. *enterica* subsp. *diarizonae* 47: k:z35* | snake | 2 |
| *S*. *enterica* subsp. *diarizonae* 57: k:e,n,x,z15* | snake | 1 |
| *S*. *enterica* subsp. *enterica* 13,23: i :- | chicken | 4 |
| *S*. *enterica* subsp. *houtenae* 51 : z4,z23 - * | snake | 1 |
| *S*. *enterica* subsp. *salamae* | snake | 1 |
| *S*. Gaminara | chicken | 2 |
| *S*. Hadar | chicken | 3 |
| *S.* Kisaware | chicken | 1 |

| Serovar | Animal | Number |
|---|---|---|
| *S.* Luckenwalde | gecko | 1 |
| *S*. Muenchen | snake | 1 |
| *S*. Mbandaka | chicken | 2 |
| *S.* Midway | chicken | 1 |
| *S.* Montevideo | chicken | 2 |
| *S.* Ndolo | chicken | 1 |
| *S.* Oranienburg* | snake | 1 |
| | dog | 1 |
| *S.* Rissen | swine | 1 |
| *S.* Senftenberg | chicken | 1 |
| *S.* Tennessee | chicken | 3 |
| *S.* Typhimurium | cattle | 19 |
| | chicken | 23 |
| | dog | 1 |
| | sheep | 1 |
| | horse | 1 |
| | parrot | 1 |
| *S.* Typhimurium, monophasic variant (4,12 : i : -) | cattle | 9 |
| | chicken | 1 |
| | horse | 1 |
| *S.* Welikate | chicken | 2 |
| No salmonella | | 2 |
| | **Total** | **129** |

* serotyping in human reference laboratory

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

| Biovar | apx group | Serotype | number |
|---|---|---|---|
| Biovar I | apx group: I BD + II CA | 7,12 | 28 |
| Biovar I | apx group: I BD + II CA + III CA + BD<br>*cps*2 gene positive | 2 | 2 |
| Biovar I | apx group: II CA + III CA + BD | 3 | 3 |
| Biovar I | apx group: III CA + BD | 3 variant | 2 |
| Biovar II | apx group: I BD + II CA<br>*cps*2 gene positive | 2 | 10 |
| No APP | - | - | 2 |
| | | **Total** | **47** |

## 3.4. Diagnostic Activity for the Border Veterinary Control

| Seafood | Total | negative | positive |
|---|---|---|---|
| Detection of *Salmonella* spp. | 11 | 11 | 0 |

These analyses will not be the task within the reference function of the ZOBA in the future.

## 3.5. Research, Development and Validation

### 3.5.1. Virulence-associated gene pattern of porcine and human *Yersinia enterocolitica* biotype 4 isolates

*Yersinia enterocolitica* (*Y. enterocolitica)* bioserotype 4/O:3 is the most important human pathogenic bioserotype in Europe and the predominant pathogenic bioserotype in slaughter pigs. Although many studies on the virulence of *Y. enterocolitica* strains have revealed a broad spectrum of detectable factors in pigs and humans, an analysis based on a strict comparative approach and serving to verify the virulence capability of porcine *Y. enterocolitica* as a source for human yersiniosis is lacking. Therefore, in the present study, strains of biotype (BT) 4 isolated from Swiss slaughter pig tonsils and feces and isolates from human clinical cases were compared in terms of their spectrum of virulence-associated genes (*yadA, virF, ail, inv, rovA, ymoA, ystA, ystB* and *myfA*). An analysis of the associated antimicrobial susceptibility pattern completed the characterization. All analyzed BT 4 strains showed a nearly similar pattern, comprising the known fundamental virulence-associated genes *yadA, virF, ail, inv, rovA, ymoA, ystA and myfA*. Only *ystB* was not detectable among all analyzed isolates. Importantly, neither the source of the isolates (porcine tonsils and feces, humans) nor the serotype (ST) had any influence on the gene pattern. From these findings, it may be concluded that all porcine BT 4 strains possess all relevant virulence genes necessary for human infection. Swiss porcine BT 4 strains showed susceptibility not only to chloramphenicol, cefotaxime, ceftazidime, ciprofloxacin, colistin, florfenicol, gentamicin, kanamycin, nalidixic acid, sulfamethoxazole, streptomycin, tetracycline and trimethoprim but also showed 100% resistance to ampicillin. The human BT 4 strains revealed comparable results. However, in addition to 100% resistance to ampicillin, 2 strains were resistant to chloramphenicol and nalidixic acid. Additionally, 1 of these strains was resistant to sulfamethoxazole.
The results demonstrated that *Y. enterocolitica* BT 4 isolates from porcine tonsils, as well as from feces, show the same virulence-associated gene pattern and resistance properties as human isolates from clinical cases, confirming the etiological role of porcine BT 4 in human yersiniosis. Thus, cross-contamination of carcasses and organs at slaughter with porcine *Y. enterocolitica* BT 4 strains, either from tonsils or feces, must be prevented to reduce human yersiniosis.

Publication: Schneeberger M, Brodard I, Overesch G. (2015) Int J Food Microbiol. Apr 2;198:70-4.

### 3.5.2. Pododermatitis in Captive and Free-Ranging Greater Flamingos (*Phoenicopterus roseus*).

Pododermatitis is frequent in captive flamingos worldwide, but little is known about the associated histopathologic lesions. Involvement of a papillomavirus or herpesvirus has been suspected. Histopathologic evaluation and viral assessment of biopsies from 19 live and 10 dead captive greater flamingos were performed. Selected samples were further examined by transmission electron microscopy and immunohistochemistry. Feet from 10 dead free-ranging greater flamingos were also evaluated. The histologic appearance of lesions of flamingos of increasing age was interpreted as the progression of pododermatitis. Mild histologic lesions were seen in a 3-week-old flamingo chick with no macroscopic lesions, and these were characterized by Micrococcus-like bacteria in the stratum corneum associated with exocytosis of heterophils. The inflammation associated with these bacteria may lead to further histologic changes: irregular columnar proliferations, papillary squirting, and dyskeratosis. In more chronic lesions, hydropic degeneration of keratinocytes, epidermal hyperplasia, and dyskeratosis were seen at the epidermis, as well as proliferation of new blood vessels and increased intercellular matrix in the dermis. Papillomavirus DNA was not identified in any of the samples, while herpesvirus DNA was seen only in a few cases; therefore, these viruses were not thought to be the cause of the lesions. Poor skin health through suboptimal husbandry may weaken the epidermal barrier and predispose the skin to invasion of Micrococcus-like bacteria. Histologic lesions were identified in very young flamingos with no macroscopic lesions; this is likely to be an early stage lesion that may progress to macroscopic lesions.

Publication: Wyss F., Schumacher V., Wenker C., Hoby S., Gobeli S., Arnaud A., Engels M., Friess M., Lange C.E., Stoffel M.H., Robert N. (2015) Vet Pathol. Nov;52:1235-42.

### 3.5.3. Comprehensive analysis of bovine abortion combining classical and next generation sequencing approaches.

Abortion in ruminants has significant economic impact in agriculture requiring prompt diagnosis of possible causes. The etiology of abortion can be very complex and the majority of abortions are not epizootic but sporadic. Yet, few infectious agents are examined in routine abortion diagnosis. In microbiome research next generation sequencing (NGS) has become a valuable tool in microbial community studies. To improve our knowledge of the bacterial community present in bovine abortion, we studied 33 placentae and 48 fetal abomasa corresponding to 63 cases by Illumina MiSeq 16S rDNA sequencing technology (Microsynth, Switzerland).
DNA extraction was carried out using the QIAamp Mini Kit (Qiagen GmbH) and amplicon sequencing targeted variable regions V3-V5 of the 16S rDNA. Samples were also analyzed by histopathology, bacteriological and mycological culture, by molecular methods for Chlamydia abortus, Coxiella burnetii and Leptospira spp. and for neosporosis, bovine viral diarrhea and Schmallenberg virus.
NGS data analysis identified an average of 30 representative operational taxonomic units (OTUs) for placenta samples (0.3% *Actinobacteria*, 5.6% *Bacteroidetes*, 16.3% *Firmicutes*, 2.8% *Fusobacteria* and 74.6% *Proteobacteria*) and 26 representative OTUs for abomasum samples (0.0009% *Actinobacteria*, 2.1% *Bacteroidetes*, 12.3%

*Firmicutes*, 0.006% Fusobacteria and 85.6% *Proteobacteria*). In both organs *Pseudomonadaceae* was the most abundant family. The results revealed opportunistic pathogens of interest such as *Campylobacter fetus* subsp. *fetus*, *Trueperella pyogenes*, *Streptococcus pluranimalium*. PCR was negative except from one case of C. burnetii. In 20 cases we found a virological (BVDV n=3, Schmallenberg n=11) or parasitological (Neospora caninum n=6) cause. All cases of neosporosis and one case of BVD, with co-infection with S. pluranimalium, showed necrotising placentitis. Broad spectrum culture revealed a possible bacterial or fungal agent in 23 cases; however, only 9 cases were associated to necrotic placentitis (Aspergillus fumigatus n=1, Candida sp. n =1, C. fetus subsp. fetus n=1, E. coli n =2, S. pluranimalium n =2, S. uberis n=2). Allover, broad spectrum analysis could attribute a possible cause of abortion in 46% of the cases.
Increased knowledge of the microbiome involved in abortion offers new perspectives in diagnosis, e.g. the implication of Pseudomonadaceae, which are known to be possible opportunists in abortion, but have not been studied in detail. The placenta remains an understudied organ and amplicon sequencing data have the potential to shed light on the relation between placental and fetal microbiome. NGS should be extended to other microorganisms such as fungi. Nonetheless, to date classical diagnostic methods are still needed to detect specific abortive agents.

Oral presentation: Vidal S., Brodard I., Posthaus H., Perreten V., Rodriguez-Campos S. (2015) XVII International Symposium of the World Association of Veterinary Laboratory Diagnosticians, Saskatoon (Canada), 15$^{th}$-18$^{th}$ June 2015.

### 3.5.4. Abortions and causes of abortion in small ruminants.

The possible reasons for reduced reproduction rates in sheep, goat and deer farming caused by abortion, stillbirth and weak offspring are numerous. However, the applied diagnostic methods are limited due to high costs. An overview of causes of abortion with focus on the bacterial agents should enlighten the topic and enhance the willingness to submit cases of abortion to the laboratory for clarification.

Publication: Rodriguez Campos S. [Abortions and causes of abortion in small ruminants. de/fr]. Forum Kleinwiederkäuer (Switzerland) 11/2015.

# 4 Publications

## 4.1. Peer-Reviewed Publications

### Publication List


Aebi M., van den Borne B.H., Raemy A., Steiner A., Pilo P., Bodmer M.. (2015) *Mycoplasma bovis* infections in Swiss dairy cattle: a clinical investigation. Acta Vet Scand. **57**:10.
Doi: 10.1186/s13028-015-0099-x.

Aye R., Kiogora M., Frey J., Pilo P., Jores J. and Naessen J. (2015) Cyto-adherence of *Mycoplasma mycoides* subsp. *mycoides* to bovine lung epithelial cells. BMC Veterinary Research **11**: 27.
Doi: 10.1186/s12917-015-0347-3

Binder A. & Lavia M., Gobeli S., Piersigilli A., Busenbach K., Schoon H.A., G. Hirsbrunner (2015) Befunde von Uterus und Ovarien geschlachteter Eringerkühe mit Fruchtbarkeitsproblemen. *Schweiz Arch Tierheilkd*. **157**: 331 – 337
PMID: 26753348

Blackburn J.K., Odugbo M.O., Van Ert M., O'Shea B., Mullins J., Perreten V., Maho A., Hugh-Jones M., Hadfield T. *Bacillus anthracis* diversity and geographic potential across Nigeria, Cameroon and Chad: further support of a novel West African lineage. (2015) PLoS Negl Trop Dis. **9**(8):e0003931.
Doi: 10.1371/journal.pntd.0003931.

Bürki, S., Frey, J. and Pilo, P. (2015) Virulence, persistence and dissemination of *Mycoplasma bovis.* Vet. Microbiol. **179**: 15–22.
Doi: org/10.1016/j.vetmic.2015.02.024

Bürki S., Gaschen V., Stoffel M.H., Stojiljkovic A., Frey J., Kuehni-Goghenbor K. and Pilo P. (2015) Invasion and persistence of *Mycoplasma bovis* in embryonic calf turbinate cells. *Veterinary Research*, **46**:53
Doi: 10.1186/s13567-015-0194-z

Carattoli A., Seiffert S.N., Schwendener S., Perreten V., Endimiani A. (2015) Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. PLoS One. **10**(5):e0123063.
Doi: 10.1371/journal.pone.0123063.

Dobay A., Pilo P., Lindholm A.K., Origgi F., Bagheri H.C., König B. (2015) Dynamics of a Tularemia Outbreak in a Closely Monitored Free-Roaming Population of Wild House Mice. PLoS One. **10**(11):e0141103.
Doi: 10.1371/journal.pone.0141103

Dreyer, M., Thomann, A., Böttcher, S., Frey, J. and Oevermann, A. (2015) Outbreak investigation identifies a single *Listeria monocytogenes* strain in sheep with different clinical manifestations, soil and water. Vet. Microbiol. **179**:69–75.
Doi: 10.1016/j.vetmic.2015.01.025.

Emond-Rheault J.G., Vincent A.T., Trudel M.V., Frey J., Frenette M. and Charette S.J. (2015). AsaGEI2b: a new variant of a genomic island identified in the *Aeromonas salmonicida* subsp. *salmonicida* JF3224 strain isolated from a wild fish in Switzerland. FEMS Microbiol Lett. **362** (13).
Doi: 10.1093/femsle/fnv093

Frey, J. and Falquet, L. (2015) Patho-genetics of Clostridium chauvoei. Research in Microbiology 166: 384-392.
Doi: 10.1016/j.resmic.2014.10.013

Ernst M., Pilo P., Fleisch F., Glisenti P. (2015) Tularemia in the Southeastern Swiss Alps at 1,700 m above sea level. Infection. **43**(1):111-5.
Doi: 10.1007/s15010-014-0676-3.

Filioussis, G., Petridou, E., Karavanis, E. and Frey, J. (2015) Pyogranulomatous pneumonia in goats caused by an undescribed *Porphyromonas* species: '*Porphyromonas katsikii*'. J. Clin. Microbiol. **53**:795-798.
Doi:2015 JCM.02682-14.

Fischer A., Santana-Cruz I., Hegerman J., Gourlé H., Schieck E., Lambert M., Nadendla S., Wesonga H., Miller R.A., Vashee S., Weber J., Meens J., Frey J, and Jores J. (2015) High quality draft genomes of the *Mycoplasma mycoides* subsp. *mycoides* challenge strains Afadé and B237. Standards in Genomic Sciences **10**: 89.
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Frey, J. and Falquet, L. (2015) Patho-genetics of *Clostridium chauvoei*. Research in Microbiology 166: 384-392.
Doi: 10.1016/j.resmic.2014.10.013

Frey, J. and Kuhnert, P. (2015) Identification of animal *Pasteurellaceae* by MALDI-TOF mass spectrometry. Methods in molecular biology **1247**:235-243.
Doi: 10.1007/978-1-4939-2004-4_18.

Gómez-Sanz E, Schwendener S, Thomann A, Gobeli Brawand S, Perreten V. (2015) First Staphylococcal Cassette Chromosome *mec* containing a *mecB*-carrying gene complex independent of transposon Tn*6045* in a *Macrococcus caseolyticus* isolate from a canine infection. Antimicrob Agents Chemother. **59**(8):4577-83.
Doi: 10.1128/AAC.05064-14.

Guldimann C., Bärtschi M., Frey J., Zurbriggen A., Torsten Seuberlich T. and Oevermann A. (2015) Increased spread and replication efficiency of *Listeria monocytogenes* in organotypic brain-slices is related to multilocus variable number of tandem repeat analysis (MLVA) complex. BMC Microbiology **15**:134.
Doi: 10.1186/s12866-015-0454-0

Henke D., Rupp S., Gaschen V., Stoffel M.H., Frey J., Vandevelde M., Oevermann A. (2015) *Listeria monocytogenes* spreads within the brain by actin-based intra-axonal migration. Infection and Immunity **83**: 2409-2419.
Doi: 10.1128/IAI.00316-15

Jonas R., Kittl S., Overesch G., Kuhnert P. (2015) Genotypes and antibiotic resistance of bovine Campylobacter and their contribution to human campylobacteriosis. Epidemiol Infect. **143**:2373-2380.
Doi: 10.1017/S0950268814003410.

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Doi: 10.1099/ijs.0.000089

Liljander A., Yu M., O'Brien E., Heller M., Nepper J.F., Weibel D.B., Gluecks I., Younan M., Frey J., Falquet L. and Jores J. (2015) A field applicable recombinase polymerase amplification assay for rapid detection of *Mycoplasma capricolum* subsp. *capripneumoniae*. J. Clin. Microbiol. **53**:2810-2815.
Doi: 10.1128/JCM.00623-15

Longo M.V., Jaton K., Pilo P., Chabanel D., Erard V. (2015) Long-Lasting Fever and Lymphadenitis: Think about *F. tularensis*. Case Rep Med. 2015:191406.
Doi: 10.1155/2015/191406.

Mulungo, M., Frey, J., Smith, K., Schnier, C., Wesonga, H., Naessens, J. and McKeever, D. (2015) Vaccination of cattle with the LppQ-N′ sub-unit of *Mycoplasma mycoides* subsp. *mycoides* results in type III immune complex disease upon experimental infection. Infect. Immun. **83**:1992-2000
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Nicklas W., Bisgaard M., Aalbæk B., Kuhnert P., Christensen H. Reclassification of *Actinobacillus muris* as *Muribacter muris* gen. nov., comb. nov. Int J Syst Evol Microbiol. **65**:3344-3351.
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Origgi F.C., König B., Lindholm A.K., Mayor D., Pilo P. (2015) Tularemia among free-ranging mice without infection of exposed humans, Switzerland, 2012. Emerg Infect Dis. **21**(1):133-5.
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Origgi F.C., Tecilla M., Pilo P., Aloisio F., Otten P., Aguilar-Bultet L., Sattler U., Roccabianca P., Romero C.H., Bloom D.C., Jacobson E.R. (2015) A Genomic Approach to Unravel Host-Pathogen Interaction in Chelonians: The Example of Testudinid Herpesvirus 3. PLoS One. **10**(8):e0134897.
Doi: 10.1371/journal.pone.0134897

Pilo P., Vogt D., Origgi F.C., Endimiani A., Peterson S., Perreten V. (2015) First report of a multidrug-resistant *Klebsiella pneumoniae* of sequence type 11 causing sepsis in a free-ranging beaver (*Castor fiber*). Environ Microbiol Rep. **7**(2):351-3.
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Doi:10.1371/journal.pone.0116730
* Associated researcher

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* Associated researcher

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* Associated researcher

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## 5. Organization chart (Organigramm)