b UNIVERSITÄT BERN

ANNUAL REPORT 2017

Institute of Veterinary Bacteriology

Vetsuisse Faculty

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1 Preface

The Institute of Veterinary Bacteriology is delighted to distribute its face-lifted Annual Report summarizing the activities and publications of 2017. This report includes more information than previous issues. It features the achievements of our staff, students and collaborators.

As in 2016, the research was mainly conducted under the funding umbrellas of major grants devoted to research on *Mycoplasma*, *Listeria*, *Dichelobacter*, abortion, antimicrobial resistance and molecular epidemiology. We contributed towards 49 peer-reviewed publications. Additionally, we started to build up a new research team utilizing synthetic biology tools to develop novel vaccines and to decipher host-pathogen interactions. In that respect we received a competitive research grant from IDRC in Canada that will enable us to increase the activities and the research staff within the two coming years.

With respect to our teaching commitments we taught students of the VETSUISSE and the SCIENCE faculty at the University of Bern and examined the latter orally. Besides the undergraduate education, we also contributed to postgraduate education, which is reflected by four veterinary master degrees and four DVM degrees. Sabrina Nathalie Andreis, who worked on the Identification of β-lactam and macrolide resistance genes in coagulase-negative staphylococci and was supervised by Vincent Perreten and Sybille Schwendener won the Faculty Price for best thesis in 2017. The staff member Sonja Kittl successfully passed the exam of the American College of Veterinary Microbiologists (ACVM) and received her diploma.

Finally, we contributed 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. In 2017, we reintegrated the development of diagnostic assays within the division of host-pathogen inteactions under the supervision of the research team of Peter Kuhnert. I thank all members of the institute, its partners, and customers who made 2017 again a successful year for veterinary bacteriology in Bern.

Bern 15th August 2018 Jörg Jores

2 Research Units

2.1 Host-Pathogen Interactions

2.1.1 Splenitis in dogs

Publication: Ferri F, Zini E, Auriemma E, Castagnaro M, Coppola LM, Peano A, Martella V, Decaro N, Kuhnert P, Ferro S. 2017. Vet Pathol **54**:147-154, *doi:* 10.1177/0300985816653989.

Collaborators: Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland; Istituto Veterinario di Novara, Granozzo con Monticello, Italy

Abstract: Splenitis is uncommonly reported in dogs. Herein, the authors describe its prevalence, clinical findings and outcomes, histologic patterns, and causes. Splenic samples of dogs diagnosed with splenitis between 2005 and 2013 were collected and stained with hematoxylin and eosin, Gram, green-Gram, Giemsa, periodic acid–Schiff, and Ziehl-Neelsen. Samples were processed for polymerase chain reaction (PCR) to detect bacteria, fungi, and protozoa (*Leishmania infantum*, *Hepatozoon canis*). Thirty-three of 660 splenic samples (5%) had splenitis. Clinical findings and outcomes were available in 19 dogs (58%); 49% had weakness, 33% had fever, and 84% survived. The most frequent inflammatory patterns included purulent splenitis (27%), pyogranulomatous splenitis (24%), and neutrophilic perisplenitis (15%). One dog had a putative diagnosis of primary splenitis; in 8 dogs, microorganisms were identified histologically or by PCR in the spleen without obvious comorbidities. Twenty-four dogs (73%) had concurrent diseases; a permissive role in the development of splenitis was suspected in 21 of these cases. Histologic examination identified the cause of splenitis in 10 dogs. Bacteria were identified by PCR in 23 cases, but the bacteria were confirmed histologically in only 6 of these. *Leishmania* was detected with PCR in 6 dogs. *Leishmania* was identified in 1 dog and H. canis in another histologically, but both were PCR negative. Fungi were identified in 8 spleens by PCR and in 1 by histology. This study suggests that splenitis is uncommon in dogs and is frequently associated with systemic diseases. Prognosis is favorable in most cases. Identification of bacteria, fungi, and protozoa in the spleens of affected dogs with PCR should be interpreted cautiously, because the findings are not confirmed histologically in many cases.

2.1.2 Occurrence of *Mycoplasma hyorhinis* infections in fattening pigs and association with clinical signs and pathological lesions of Enzootic Pneumonia

Publication: Luehrs A, Siegenthaler S, Grutzner N, Grosse Beilage E, Kuhnert P, Nathues H. 2017. Vet Microbiol **203**:1-5, *doi:* 10.1016/j.vetmic.2017.02.001.

Collaborators: Clinic for Swine, Vetsuisse Faculty, University of Bern, Switzerland; Field Station for Epidemiology, University of Veterinary Medicine Hannover, Germany

Abstract: Respiratory disorders in fattening pigs are of major concern worldwide. Particularly Enzootic Pneumonia remains a problem for the pig industry. This chronic respiratory disease is primarily caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*). However, more recently it was hypothesised that *M. hyorhinis* can also cause similar lung lesions. To investigate the relevance of *M. hyorhinis* as a cause of pneumonia in fattening pigs 10 farms in Switzerland (considered free of Enzootic Pneumonia) and 20 farms in Germany (regarded as endemic for

Enzootic Pneumonia) with a history of chronic and/or recurrent respiratory diseases were included in the study. During a one-time farm visit the coughing index was determined in the batch of oldest fattening pigs in each farm before submission to slaughter. In total, 1375 lungs from these pigs were collected at the abattoir and individually scored for lesions. Furthermore, 600 lungs with, if present, indicative lesions for Enzootic Pneumonia (purple to grey areas of tissue consolidation in the cranio-ventral lung lobes) were tested for mycoplasma species by culture and by real-time PCR for the presence of *M. hyorhinis* and *M. hyopneumoniae*. In total, 15.7% of the selected lungs were tested positive for *M. hyorhinis* by real-time PCR. The prevalence of *M. hyorhinis* was 10% in Switzerland and 18.5% in Germany and differed significantly between these two countries ($p = 0.007$). *M. hyorhinis* was detected significantly more often in pneumonic lungs ($p = 0.004$) but no significant association was found between *M. hyorhinis* and the coughing index or the *M. hyopneumoniae* status of the pig. *M. hyopneumoniae* was detected in 0% and 78.5% of the selected lungs in Switzerland and Germany, respectively. We found no evidence that *M. hyorhinis* alone can lead to similar lung lesions as seen by an infection with *M. hyopneumoniae* in fattening pigs. In addition, a simultaneous infection with both *M. hyorhinis* and *M. hyopneumoniae* did not aggravate the observed lung lesions. Moreover, the presence of *M. hyorhinis* showed no clinical effect in terms of coughing at least at the end of the fattening phase. However, different levels of virulence of *M. hyorhinis* isolates as well as interactions with viral pathogens like porcine reproductive and respiratory syndrome virus (PRRSV) or porcine circovirus type 2 (PCV2) were reported in the literature and need to be further investigated.

2.1.3 ***Rodentibacter*** gen. nov. including ***Rodentibacter pneumotropicus*** comb. nov., ***Rodentibacter heylii*** sp. nov., ***Rodentibacter myodis*** sp. nov., ***Rodentibacter ratti*** sp. nov., ***Rodentibacter heidelbergensis*** sp. nov., ***Rodentibacter trehalosifermentans*** sp. nov., ***Rodentibacter rarus*** sp. nov., ***Rodentibacter mrazii*** and two genomospecies.

Publication: Adhikary S, Nicklas W, Bisgaard M, Boot R, Kuhnert P, Waberschek T, Aalbaek B, Korczak B, Christensen H. 2017. Int J Syst Evol Microbiol **67**:1793-1806, *doi:* 10.1099/ijsem.0.001866.

Collaborators: Department of Veterinary Disease Biology, University of Copenhagen, Denmark

Abstract: Rodentibacter gen. nov. is proposed based on isolation and phenotypic characterization of strains, predominantly from rodents. The strains showed 86% or higher *rpoB* gene sequence similarity and indicated a genus-level relationship within *Pasteurellaceae*. The strains compared at 16S rRNA gene sequence level showed 93.8% or higher similarity, and their genus-level relationship within *Pasteurellaceae* was confirmed by phenotypic analysis. The type species *Rodentibacter pneumotropicus* comb. nov. is reclassified from [*Pasteurella*] *pneumotropica* with type strain NCTC 8141T (=CCUG 12398T). Whole genomic comparison allowed the estimation of DNA–DNA renaturation. *Rodentibacter heylii* sp. nov. was proposed for a group that included the biovar Heyl of [*Pasteurella*] *pneumotropica* with the type strain ATCC 12555T (=CCUG 998T). A group was proposed as *Rodentibacter ratti* sp. nov., which

included the taxon 22 of Bisgaard; the type strain is F75T (=CCUG 69665T=DSM 103977T). Taxon 41 of Bisgaard was proposed as *Rodentibacter myodis* sp. nov. with type strain Ac151T (=CCUG 69666T=DSM 103994T). *Rodentibacter heidelbergensis* sp. nov. included the type strain 1996025094T (=Ac69T) (=CCUG 69667T=DSM 103978T). A group strains of was proposed as *Rodentibacter trehalosifermentans* sp. nov. with type strain H1987082031T (=CCUG 69668T=DSM 104075T). Two strains including the reference strain of taxon 17 of Bisgaard that showed 16S rRNA gene similarity of 97.3% were proposed as *Rodentibacter rarus* sp. nov. 2325/79T (=CCUG 17206T=DSM 103980T). *Rodentibacter mrazii* sp. nov. was proposed with type strain Ppn418T (Bisgaard taxon 21) (=CCUG 69669T=DSM 103979T). The eight species could be separated based on phenotypic characteristics. Forty-six strains including taxon 48 of Bisgaard formed a monophyletic group by *rpoB* and 16S rRNA gene sequence analysis, but could not be separated phenotypically from *R. pneumotropicus* and *R. heylii*, and it was left as an unnamed genomospecies 1 of *Rodentibacter* with reference strain Ppn416. Another taxon that included 13 strains, mainly isolated from *Apodemus sylvaticus*, could not be separated phenotypically from *R. pneumotropicus* or *R. heylii* and was designated as genomospecies 2. Strain Ppn85 with 95% or less *rpoB* gene sequence similarity and with 16S rRNA gene sequence similarity of 97% or less to the other members of *Rodentibacter* was left as an unnamed singleton.

2.1.4 Differential Infection Patterns and Recent Evolutionary Origins of Equine Hepaciviruses in Donkeys

Publication: Walter S, Rasche A, Moreira-Soto A, Pfaender S, Bletsa M, Corman VM, Aguilar-Setien A, Garcia-Lacy F, Hans A, Todt D, Schuler G, Shnaiderman-Torban A, Steinman A, Roncoroni C, Veneziano V, Rusenova N, Sandev N, Rusenov A, Zapryanova D, Garcia-Bocanegra I, Jores J, Carluccio A, Veronesi MC, Cavalleri JM, Drosten C, Lemey P, Steinmann E, Drexler JF. 2017. J Virol **91**, *doi:* 10.1128/JVI.01711-16.

Collaborators: Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

Abstract: The hepatitis C virus (HCV) is a major human pathogen. Genetically related viruses in animals suggest a zoonotic origin of HCV. The closest relative of HCV is found in horses (termed equine hepacivirus [EqHV]). However, low EqHV genetic diversity implies relatively recent acquisition of EqHV by horses, making a derivation of HCV from EqHV unlikely. To unravel the EqHV evolutionary history within equid sister species, we analyzed 829 donkeys and 53 mules sampled in nine European, Asian, African, and American countries by molecular and serologic tools for EqHV infection. Antibodies were found in 278 animals (31.5%), and viral RNA was found in 3 animals (0.3%), all of which were simultaneously seropositive. A low RNA prevalence in spite of high seroprevalence suggests a predominance of acute infection, a possible difference from the mostly chronic hepacivirus infection pattern seen in horses and humans. Limitation of transmission due to short courses of infection may explain the existence of entirely seronegative groups of animals. Donkey and horse EqHV strains were paraphyletic and 97.5 to 98.2% identical in their translated polyprotein sequences, making virus/host cospeciation unlikely. Evolutionary reconstructions supported host switches of

EqHV between horses and donkeys without the involvement of adaptive evolution. Global admixture of donkey and horse hepaciviruses was compatible with anthropogenic alterations of EqHV ecology. In summary, our findings do not support EqHV as the origin of the significantly more diversified HCV. Identification of a host system with predominantly acute hepacivirus infection may enable new insights into the chronic infection pattern associated with HCV.

IMPORTANCE: The evolutionary origins of the human hepatitis C virus (HCV) are unclear. The closest animal-associated relative of HCV occurs in horses (equine hepacivirus [EqHV]). The low EqHV genetic diversity implies a relatively recent acquisition of EqHV by horses, limiting the time span for potential horse-to-human infections in the past. Horses are genetically related to donkeys, and EqHV may have cospeciated with these host species. Here, we investigated a large panel of donkeys from various countries using serologic and molecular tools. We found EqHV to be globally widespread in donkeys and identify potential differences in EqHV infection patterns, with donkeys potentially showing enhanced EqHV clearance compared to horses. We provide strong evidence against EqHV cospeciation and for its capability to switch hosts among equines. Differential hepacivirus infection patterns in horses and donkeys may enable new insights into the chronic infection pattern associated with HCV.

2.1.5 Development of field-applicable tests for rapid and sensitive detection of *Candidatus* Phytoplasma oryzae

Publication: Wambua L, Schneider B, Okwaro A, Wanga JO, Imali O, Wambua PN, Agutu L, Olds C, Jones CS, Masiga D, Midega C, Khan Z, Jores J, Fischer A. 2017. Mol Cell Probes **35**:44-56, *doi:* 10.1016/j.mcp.2017.06.004.

Collaborators: icipe, Nairobi, Kenya

Abstract: Napier grass Stunt Disease (NSD) is a severe disease of Napier grass (*Pennisetum purpureum*) in Eastern Africa, caused by the leafhopper-transmitted bacterium *Candidatus* Phytoplasma oryzae. The pathogen severely impairs the growth of Napier grass, the major fodder for dairy cattle in Eastern Africa. NSD is associated with biomass losses of up to 70% of infected plants. Diagnosis of NSD is done by nested PCR targeting the phytoplasma DNA, which is difficult to perform in developing countries with little infrastructure. We report the development of an easy to use, rapid, sensitive and specific molecular assay for field diagnosis of NSD. The procedure is based on recombinase polymerase amplification and targets the *imp* gene encoding a pathogen-specific immunodominant membrane protein. Therefore, we followed a two-step process. First, we developed an isothermal DNA amplification method for real time fluorescence application and then transferred this assay to a lateral flow format. The limit of detection for both procedures was estimated to be 10 organisms. We simplified the template preparation procedure by using freshly squeezed phloem sap from Napier grass. Additionally, we developed a laboratory serological assay with the potential to be converted to a lateral flow assay. Two murine monoclonal antibodies with high affinity and specificity to the immunodominant membrane protein IMP of *Candidatus* Phytoplasma oryzae were generated. Both antibodies specifically reacted with the denatured or native 17 kDa IMP protein. In dot

blot experiments of extracts from infected plant, phytoplasmas were detected in as little as 12.5 μg of fresh plant material.

2.1.6 Temporal induction of pro-inflammatory and regulatory cytokines in human peripheral blood mononuclear cells by *Campylobacter jejuni* and *Campylobacter coli*

Publication: Hamza, E., Kittl, S., Kuhnert, P. (2017) PLoS ONE **12**: e0171350

Collaborators: Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Egypt

Campylobacter jejuni along with *C. coli* are major cause of human gastroenteritis worldwide. So far, the human immune response against *Campylobacter* is not entirely clear. We hypothesize that it is coordinated by an interaction between pro-inflammatory and regulatory cytokines which is influenced by bacterial and host-individual differences. Accordingly, we used peripheral blood mononuclear cells (PBMC) from healthy donors to study the primary systemic immune response to *C. jejuni* and *C. coli*. PBMC were stimulated by different strains of *C. jejuni* and *C. coli* for three time points (5, 10, 24 hours). The production of the pro-inflammatory (IL-6, IL-8, IFN-γ) and the regulatory (IL-10) cytokines were measured by ELISA. All strains induced higher levels of IL-8 and IL-6 than IFN-γ and IL-10. In contrast to IL-8 and IL-6, IL-10 showed a steeper increase over time. While IFN-γ did not show any further increase between 10 and 24 hours. Interestingly, there was a significant correlation between IL-8 and IL-10 which peaked at 24 hours. Despite the variability of the used bacterial strains, their effect on cytokine production was less pronounced than the inter-person differences. The strongest significant effect of the strain was on the level of IL-10. IL-10 and IL-6 were significantly influenced by strain-person interaction. In conclusion, the systemic immune response to *C. coli* and *C. jejuni* is characterized by an early pro-inflammatory reaction with later initiation of regulatory immune response which is influenced mainly by the host, explaining the individual variations in disease severity. Additional work is needed to determine the cellular sources of the produced cytokines as well as the campylobacter molecules that might contribute to this stimulation.

2.1.7 Long-term dynamics of *Mycoplasma conjunctivae* at the wildlife-livestock interface in the Pyrenees

Publication: Fernandez-Aguilar X, Cabezon O, Frey J, Velarde R, Serrano E, Colom-Cadena A, Gelormini G, Marco I, Mentaberre G, Lavin S, Lopez-Olvera JR. 2017. PLoS One **12**:e0186069, *doi:* 10.1371/journal.pone.0186069.

Collaborators: Universitat Autonoma de Barcelona, Bellaterra, Spain; Universidad de Aveiro, Portugal

Abstract: Functional roles of domestic and wild host populations in infectious keratoconjunctivitis (IKC) epidemiology have been extensively discussed claiming a domestic reservoir for the more susceptible wild hosts, however, based on limited data. With the aim to better assess IKC epidemiology in complex host-pathogen alpine systems, the long-term

infectious dynamics and molecular epidemiology of *Mycoplasma conjunctivae* was investigated in all host populations from six study areas in the Pyrenees and one in the Cantabrian Mountains (Northern Spain). Detection of *M. conjunctivae* was performed by qPCR on 3600 eye swabs collected during seven years from hunted wild ungulates and sympatric domestic sheep (n = 1800 animals), and cluster analyses of the strains were performed including previous reported local strains. *Mycoplasma conjunctivae* was consistently detected in three Pyrenean chamois (*Rupicapra p. pyrenaica*) populations, as well as in sheep flocks (17.0% of sheep) and occasionally in mouflon (*Ovis aries musimon*) from the Pyrenees (22.2% in one year/area); statistically associated with ocular clinical signs only in chamois. Chamois populations showed different infection dynamics with low but steady prevalence (4.9%) and significant yearly fluctuations (0.0%- 40.0%). Persistence of specific *M. conjunctivae* strain clusters in wild host populations is demonstrated for six and nine years. Cross-species transmission between chamois and sheep and chamois and mouflon were also sporadically evidenced. Overall, independent *M. conjunctivae* sylvatic and domestic cycles occurred at the wildlife-livestock interface in the alpine ecosystems from the Pyrenees with sheep and chamois as the key host species for each cycle, and mouflon as a spill-over host. Host population characteristics and *M. conjunctivae* strains resulted in different epidemiological scenarios in chamois, ranging from the fading out of the mycoplasma to the epidemic and endemic long-term persistence. These findings highlight the capacity of *M. conjunctivae* to establish diverse interactions and persist in host populations, also with different transmission conditions.

2.1.8 Postepizootic Persistence of Asymptomatic *Mycoplasma conjunctivae* Infection in Iberian Ibex

Publication: Fernandez-Aguilar X, Cabezon O, Granados JE, Frey J, Serrano E, Velarde R, Cano-Manuel FJ, Mentaberre G, Raez-Bravo A, Fandos P, Lopez-Olvera JR. 2017. Appl Environ Microbiol **83**, *doi:* 10.1128/AEM.00690-17.

Collaborators: Universitat Autonoma de Barcelona, Barcelona, Spain; Espacio Natural de Sierra Nevada, Pinos Genil, Granada, Spain; Universidad de Aveiro, Portugal; Agencia de Medio Ambiente y Agua, Seville, Spain

Abstract: The susceptibility of the Iberian ibex (*Capra pyrenaica*) to *Mycoplasma conjunctivae* ocular infection and the changes in their interaction over time were studied in terms of clinical outcome, molecular detection, and IgG immune response in a captive population that underwent a severe infectious keratoconjunctivitis (IKC) outbreak. *Mycoplasma conjunctivae* was detected in the Iberian ibex, coinciding with the IKC outbreak. Its prevalence had a decreasing trend in 2013 that was consistent with the clinical resolution (August, 35.4%; September, 8.7%; November, 4.3%). Infections without clinical outcome were, however, still detected in the last handling in November. Sequencing and cluster analyses of the *M. conjunctivae* strains found 1 year later in the ibex population confirmed the persistence of the same strain lineage that caused the IKC outbreak but with a high prevalence (75.3%) of mostly asymptomatic infections and with lower DNA load of *M. conjunctivae* in the eyes (mean quantitative PCR [qPCR] cycle threshold [CT], 36.1 versus 20.3 in severe IKC). Significant age-related differences of *M.*

conjunctivae prevalence were observed only under IKC epizootic conditions. No substantial effect of systemic IgG on *M. conjunctivae* DNA in the eye was evidenced with a linear mixed-models selection, which indicated that systemic IgG does not necessarily drive the resolution of *M. conjunctivae* infection and does not explain the epidemiological changes observed. The results show how both epidemiological scenarios, i.e., severe IKC outbreak and mostly asymptomatic infections, can consecutively occur by entailing mycoplasma persistence.

IMPORTANCE: *Mycoplasma* infections are reported in a wide range of epidemiological scenarios that involve severe disease to asymptomatic infections. This study allows a better understanding of the transition between two different *Mycoplasma conjunctivae* epidemiological scenarios described in wild host populations and highlights the ability of *M. conjunctivae* to adapt, persist, and establish diverse interactions with its hosts. The proportion of asymptomatic and clinical *M. conjunctivae* infections in a host population may not be regarded only in response to intrinsic host species traits (i.e., susceptibility) but also to a specific host-pathogen interaction, which in turn influences the infection dynamics. Both epidemic infectious keratoconjunctivitis and a high prevalence of asymptomatic *M. conjunctivae* infections may occur in the same host population, depending on the circulation of *M. conjunctivae*, its maintenance, and the progression of the host-pathogen interactions.

2.1.9 Infectious keratoconjunctivitis and occurrence of *Mycoplasma conjunctivae* and *Chlamydiaceae* in small domestic ruminants from Central Karakoram, Pakistan

Publication: Fernandez-Aguilar X, Rossi L, Cabezon O, Giorgino A, Victoriano Llopis I, Frey J, Lopez-Olvera JR. 2017. Vet Rec **181**:237, *doi:* 10.1136/vr.103948.

Collaborators: Universitat Autonoma de Barcelona, Bellaterra, Spain; University of Torino, Grugliasco, Italy

Abstract: Infectious keratoconjunctivitis (IKC) is a contagious eye disease primarily caused by *Mycoplasma conjunctivae* in domestic and wild *Caprinae*. *Chlamydophila* species have also been detected in ruminants with IKC. The objectives of this study are to investigate the ocular infection of *M. conjunctivae* and *Chlamydiaceae* and assess its interaction in relation to IKC in sheep and goats from remote communities around the Central Karakoram National Park in Pakistan, performing a combination of cross-sectional and case-control study design. Mostly asymptomatic and endemic infections of *M. conjunctivae* and *Chlamydiaceae* were found in sheep (19.3 per cent and 4.5 per cent, respectively) and goats (9.5 per cent and 1.9 per cent, respectively) from all communities, assessed by qPCR. Prevalence significantly differed between species only for *M. conjunctivae* ($P=0.0184$), which was also more prevalent in younger sheep ($P<0.01$). *Chlamydophila pecorum* was identified by sequencing and was related with IKC only when coinfection with M. conjunctivae occurred, which suggest a synergic interaction. Cluster analysis of *M. conjunctivae* strains revealed higher diversity of strains than expected, evidenced interspecific transmission and suggested a higher local livestock trade than previously assumed. These results highlight the widespread occurrence of *M conjunctivae* in sheep worldwide and its implications for wildlife should be assessed from a conservation perspective.

2.1.10 Infectious keratoconjunctivitis in wild *Caprinae*: merging field observations and molecular analyses sheds light on factors shaping outbreak dynamics

Publication: Gelormini G, Gauthier D, Vilei EM, Crampe JP, Frey J, Ryser-Degiorgis MP. 2017. BMC Vet Res **13**:67, *doi:* 10.1186/s12917-017-0972-0.

Collaborators: Centre for Fish and Wildlife Health (FIWI), Vetsuisse Faculty, University of Bern, Switzerland; Laboratoire Veterinaire des Hautes Alpes, Gap, France; Parc National des Pyrenees, Tarbes, France

Abstract: BACKGROUND: Infectious keratoconjunctivitis (IKC) is an ocular infectious disease caused by *Mycoplasma conjunctivae* which affects small domestic and wild mountain ruminants. Domestic sheep maintain the pathogen but the detection of healthy carriers in wildlife has raised the question as to whether *M. conjunctivae* may also persist in the wild. Furthermore, the factors shaping the dynamics of IKC outbreaks in wildlife have remained largely unknown. The aims of this study were (1) to verify the etiological role of *M. conjunctivae* in IKC outbreaks recorded between 2002 and 2010 at four study sites in different regions of France (Pyrenees and Alps, samples from 159 Alpine ibex *Capra ibex*, Alpine chamois *Rupicapra rupicapra* and Pyrenean chamois *Rupicapra pyrenaica*); (2) to establish whether there existed any epidemiological links between the different regions through a cluster analysis of the detected strains (from 80 out of the 159 animals tested); (3) to explore selected pathogen, host and environmental factors potentially influencing the dynamics of IKC in wildlife, by joining results obtained by molecular analyses and by field observations (16,609 animal observations). All of the samples were tested for *M. conjunctivae* by qPCR, and cluster analysis was based on a highly variable part of the *lppS* gene. RESULTS: We documented infections with *M. conjunctivae* in epidemic and endemic situations, both in symptomatic and asymptomatic animals. The identified *M. conjunctivae* strains were site-specific and persisted in the local wild population for at least 6 years. In epidemic situations, peaks of cases and disease resurgence were associated with the emergence of new similar strains in a given area. Social interactions, seasonal movements and the landscape structure such as natural and anthropogenic barriers influenced the spatio-temporal spread of IKC. Adults were more affected than young animals and host susceptibility differed depending on the involved strain. CONCLUSION: Our study indicates that IKC is a multifactorial disease and that *M. conjunctivae* can persist in wildlife populations. The disease course in individual animals and populations is influenced by both host and mycoplasma characteristics, and the disease spread within and among populations is shaped by host behavior and landscape structure.

2.1.11 *Aeromonas salmonicida* type III secretion system-effectors-mediated immune suppression in rainbow trout (*Oncorhynchus mykiss*)

Publication: Origgi FC, Benedicenti O, Segner H, Sattler U, Wahli T, Frey J. 2017. Fish Shellfish Immunol **60**:334-345, *doi:* 10.1016/j.fsi.2016.12.006.

Collaborators: Scottish Fish Immunology Research Centre, Institute of Biological and Environmental Sciences, University of Aberdeen, UK; Centre for Fish and Wildlife Health (FIWI), University of Bern, Switzerland

Abstract: Aeromonas salmonicida subsp. *salmonicida*, the etiologic agent of furunculosis, is a major pathogen in aquaculture. Together with other pathogens, it is characterized by the presence of a type 3 secretion system (T3SS). The T3SS is the main virulence mechanism of *A. salmonicida*. It is used by the bacterium to secrete and translocate several toxins and effector proteins into the host cell. Some of these factors have a detrimental impact on the integrity of the cell cytoskeleton, likely contributing to impair phagocytosis. Furthermore, it has been suggested that effectors of the T3SS are able to modulate the host's immune response. Here we present the first partial characterization of the immune response in rainbow trout (*Oncorhynchus mykiss*) infected with distinct strains of *A. salmonicida* either carrying (i) a fully functional T3SS or (ii) a functionally impaired T3SS or (iii) devoid of T3SS ("cured" strain). Infection with an *A. salmonicida* strain either carrying a fully functional or a secretion-impaired T3SS was associated with a strong and persistent immune suppression. However, the infection appeared to be fatal only in the presence of a fully functional T3SS. In contrast, the absence of T3SS was neither associated with immune suppression nor fish death. These findings suggest that the T3SS and T3SS-delivered effector molecules and toxins of *A. salmonicida* do not only impair the host cells' cytoskeleton thus damaging cell physiology and phagocytosis, but also heavily affect the transcription of critical immune mediators including the shut-down of important warning signals to recognize infection and induce immune defense.

2.1.12 Assessing Fifty Years of General Health Surveillance of Roe Deer in Switzerland: A Retrospective Analysis of Necropsy Reports

Publication: Pewsner M, Origgi FC, Frey J, Ryser-Degiorgis MP. 2017. PLoS One **12**:e0170338, *doi:* 10.1371/journal.pone.0170338.
Collaborators: Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Switzerland

Abstract: General wildlife health surveillance is a valuable source of information on the causes of mortality, disease susceptibility and pathology of the investigated hosts and it is considered to be an essential component of early warning systems. However, the representativeness of data from such surveillance programs is known to be limited by numerous biases. The roe deer (*Capreolus capreolus capreolus*) is the most abundant ungulate and a major game species all over Europe. Yet, internationally available literature on roe deer pathology is scarce. The aims of this study were (1) to provide an overview of the causes of mortality or morbidity observed in roe deer in Switzerland and to assess potential changes in the disease pattern over time; and (2) to evaluate the value and limitations of a long term dataset originating from general wildlife health surveillance. We compiled 1571 necropsy reports of free ranging roe deer examined at the Centre for Fish and Wildlife Health in Switzerland from 1958 to 2014. Descriptive data analysis was performed considering animal metadata, submitter, pathologist in charge, laboratory methods, morphological diagnoses and etiologies. Recurrent causes of mortality and disease pictures included pneumonia, diarrhea, meningoencephalitis, actinomycosis, blunt

trauma, predation, neoplasms and anomalies. By contrast, other diagnoses such as fatal parasitic gastritis, suspected alimentary intoxication and reproductive disorders appeared only in earlier time periods. Diseases potentially relevant for other animals or humans such as caseous lymphadenitis (or pseudotuberculosis), salmonellosis, paratuberculosis and listeriosis were sporadically observed. The disease pattern in roe deer from Switzerland was largely in accordance with previous reports. The observed fluctuations were consistent with methodical and/or personnel changes and varying disease awareness. Nevertheless, despite such limitations, the compiled data provide a valuable baseline. To facilitate comparison among studies, we recommend systematically archiving all case documents and fixed tissues and to perform data analyses more regularly and in a harmonized way.

2.1.13 Remote Sensing of Potential Biosignatures from Rocky, Liquid, or Icy (Exo)Planetary Surfaces

Publication: Poch O, Frey J, Roditi I, Pommerol A, Jost B, Thomas N. 2017. Astrobiology **17**:231-252, *doi:* 10.1089/ast.2016.1523.

Collaborators: Center for Space and Habitability, University of Bern, Switzerland; Institute of Veterinary Bacteriology, University of Bern , Switzerland; Institut fur Zellbiologie (IZB), University of Bern, Switzerland; Physikalisches Institut, University of Bern, Switzerland

Abstract: To detect signs of life by remote sensing on objects of our Solar System and on exoplanets, the characterization of light scattered by surface life material could complement possible clues given by the atmospheric composition. We reviewed the reflectance spectra of a broad selection of major biomolecules that constitute terrestrial carbon-based life from 0.4 to 2.4 µm, and we discuss their detectability through atmospheric spectral windows. Biomolecule features in the near-infrared (0.8-2.4 µm) will likely be obscured by water spectral features and some atmospheric gases. The visible range (0.4-0.8 µm), including the strong spectral features of pigments, is the most favorable. We investigated the detectability of a pigmented microorganism (*Deinococcus radiodurans*) when mixed with silica sand, liquid water, and water-ice particles representative of diverse surfaces of potentially habitable worlds. We measured the visible to near-infrared reflectance spectra (0.4-2.4 µm) and the visible phase curves (at 0.45 and 0.75 µm) of the mixtures to assess how the surface medium and the viewing geometry affect the detectability of the microorganisms. The results show that ice appears to be the most favorable medium for the detection of pigments. Water ice is bright and featureless from 0.4 to 0.8 µm, allowing the absorption of any pigment present in the ice to be well noticeable. We found that the visible phase curve of water ice is the most strongly affected by the presence of pigments, with variations of the spectral slope by more than a factor of 3 with phase angles. Finally, we show that the sublimation of the ice results in the concentration of the biological material onto the surface and the consequent increase of its signal. These results have applications to the search for life on icy worlds, such as Europa or Enceladus.

2.1.14 Hyperinvasiveness and increased intercellular spread of *Listeria monocytogenes*

sequence type 1 are independent of listeriolysin S, internalin F and internalin J1

Publication: Rupp S, Bartschi M, Frey J, Oevermann A. 2017. J Med Microbiol **66**:1053-1062, *doi:* 10.1099/jmm.0.000529.

Collaborators: Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Switzerland; Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland; Division of Neurological Sciences, Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty, University of Bern, Switzerland

Abstract: PURPOSE: *Listeria monocytogenes* is a genetically heterogeneous species, which is divided into evolutionary lineages and clonal complexes (CCs). Not all *L. monocytogenes* isolates are equally likely to cause disease, with CC1, and in particular sequence type (ST) 1, being the most prevalent complex in human and ruminant infections and more specifically in neurolisteriosis. While the major factors that determine neurotropism are unknown, the *L. monocytogenes* CC1 strains harbour listeriolysin S (*lls*) and particular alleles of internalin (*inl*) F and *inlJ*, which are not present in CCs commonly isolated from food and the environment. The aim of this study was to analyse the role of these factors in cellular infection. METHODOLOGY: A ST1 field strain (JF5203) from CC1 isolated from a bovine rhombencephalitis case was used to create deletion mutants. These were tested alongside the parental strain and EGD-e (CC9), in different culture models representing *L. monocytogenes* targets (neurons, microglia, placenta, intestine and macrophages). The phenotype was assessed by quantification of c.f.u. from cell lysates and immunofluorescence analysis. RESULTS: Compared to EGD-e, the ST1 strain JF5203 was hyperinvasive and exhibited increased intercellular spread. However, deletion of *llsB*, *inlF* or *inlJ1*, had no significant effect on infection or growth in the culture models tested. CONCLUSION: Our results underline the importance of using relevant clinical strains when investigating *L. monocytogenes* virulence. We show that despite the association with CC1, *llsB*, *inlF* and *inlJ1* are not involved in the hyperinvasiveness and efficient intercellular spread of ST1 in various cell types.

2.1.15 *Clostridium chauvoei*, an Evolutionary Dead-End Pathogen

Publication: Rychener L, InAlbon S, Djordjevic SP, Chowdhury PR, Ziech RE, de Vargas AC, Frey J, Falquet L. 2017. Front Microbiol **8**:1054, *doi:* 10.3389/fmicb.2017.01054.

Collaborators: The iThree Institute, University of Technology Sydney, UltimoNSW, Australia; Department of Preventive Veterinary Medicine, Federal University of Santa Maria, Brazil; Department of Biology, Swiss Institute of Bioinformatics, University of Fribourg, Switzerland

Abstract: Full genome sequences of 20 strains of *Clostridium chauvoei*, the etiological agent of blackleg of cattle and sheep, isolated from four different continents over a period of 64 years (1951-2015) were determined and analyzed. The study reveals that the genome of the species *C. chauvoei* is highly homogeneous compared to the closely related species *C. perfringens*, a widespread pathogen that affects human and many animal species. Analysis of the CRISPR locus is sufficient to differentiate most *C. chauvoei* strains and is the most heterogenous region

in the genome, containing in total 187 different spacer elements that are distributed as 30 - 77 copies in the various strains. Some genetic differences are found in the 3 allelic variants of *fliC1*, *fliC2* and *fliC3* genes that encode structural flagellin proteins, and certain strains do only contain one or two alleles. However, the major virulence genes including the highly toxic *C. chauvoei* toxin A, the sialidase and the two hyaluronidases are fully conserved as are the metabolic and structural genes of *C. chauvoei*. These data indicate that *C. chauvoei* is a strict ruminant-associated pathogen that has reached a dead end in its evolution.

2.1.16 A review of methods used for studying the molecular epidemiology of *Brachyspira hyodysenteriae*

Publication: Zeeh F, Nathues H, Frey J, Muellner P, Fellstrom C. 2017. Vet Microbiol **207**:181-194, *doi:* 10.1016/j.vetmic.2017.06.011.

Collaborators: Clinic for Swine, Vetsuisse Faculty, University of Bern, Switzerland; Epi-interactive, PO Box 15327, Miramar, Wellington, 6243, New Zealand; Department of Clinical Sciences, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract: Brachyspira (B.) spp. are intestinal spirochaetes isolated from pigs, other mammals, birds and humans. In pigs, seven *Brachyspira* spp. have been described, i.e. *B. hyodysenteriae*, *B. pilosicoli*, *B. intermedia*, *B. murdochii*, *B. innocens*, *B. suanatina* and *B. hampsonii*. *Brachyspira hyodysenteriae* is especially relevant in pigs as it causes swine dysentery and hence considerable economic losses to the pig industry. Furthermore, reduced susceptibility of *B. hyodysenteriae* to antimicrobials is of increasing concern. The epidemiology of *B. hyodysenteriae* infections is only partially understood, but different methods for detection, identification and typing have supported recent improvements in knowledge and understanding. In the last years, molecular methods have been increasingly used. Molecular epidemiology links molecular biology with epidemiology, offering unique opportunities to advance the study of diseases. This review is based on papers published in the field of epidemiology and molecular epidemiology of *B. hyodysenteriae* in pigs. Electronic databases were screened for potentially relevant papers using title and abstract and finally, Barcellos et al. papers were systemically selected and assessed. The review summarises briefly the current knowledge on *B. hyodysenteriae* epidemiology and elaborates on molecular typing techniques available. Results of the studies are compared and gaps in the knowledge are addressed. Finally, potential areas for future research are proposed.

2.2 Molecular and Bacterial Epidemiology and Infectious Diseases

2.2.1 *Macrococcus canis* and *M. caseolyticus* in dogs: occurrence, genetic diversity and antibiotic resistance.

Publication: Cotting K, Strauss C, Rodriguez-Campos S, Rostaher A, Fischer NM, Roosje PJ, Favrot C, Perreten V. 2017. Vet Dermatol **28**:559-e133, *doi:* 10.1111/vde.12474

Collaborators: Clinic for Small Animal Internal Medicine, Dermatology unit, Vetsuisse Faculty, University of Zurich, Switzerland; Division of Clinical Dermatology, Department of Clinical Veterinary Medicine, University of Bern, Switzerland

Abstract: The discovery of a new *Macrococcus canis* species isolated from skin and infection sites of dogs led us to question if *Macrococcus* spp. are common in dogs and are resistant to antibiotics. One hundred and sixty two dogs (mainly West Highland white terriers and Newfoundland dogs) were screened for the presence of *Macrococcus*, including six dogs with *Macrococcus* infections. Samples were taken from skin, ear canal and oral mucosa using swabs. Macrococci were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry, 16S rRNA sequencing and *nuc*-PCR. Minimal inhibitory concentrations of 19 antibiotics were determined using broth microdilution. Resistance mechanisms were identified by microarray and sequencing of the fluoroquinolone-determining region of *gyrA* and *grlA*. Sequence type (ST) was determined by multilocus sequence typing. Out of the 162 dogs, six harboured *M. caseolyticus* (n = 6) and 13 harboured *M. canis* (n = 16). Six isolates of M. canis and one of *M. caseolyticus* were obtained from infection sites. The 22 *M. canis* strains belonged to 20 different STs and the seven *M. caseolyticus* strains to three STs. Resistance to antibiotics was mostly associated with the detection of known genes, with *mecB*-mediated meticillin resistance being the most frequent. This study gives some insights into the occurrence and genetic characteristics of antibiotic-resistant *Macrococcus* from dogs. Presence of *M. canis* in infection sites and resistance to antibiotics emphasized that more attention should be paid to this novel bacteria species.

2.2.2 Pathotyping and antibiotic resistance of porcine enterovirulent *Escherichia coli* strains from Switzerland (2014-2015)

Publication: Brand P, Gobeli S, Perreten V. 2017. Schweiz Arch Tierheilkd **159**:373-380, *doi:* 10.17236/sat00120.

Collaborators: N/A

Abstract: A total of 131 porcine *E. coli* were isolated in 2014 and 2015 from the gut of 115 pigs raised in Switzerland and suffering from diarrhea. The isolates were tested for antibiotic resistance, serotypes, virulence factors and genetic diversity. Serotypes were assigned by agglutination tests and virulence genes were identified by polymerase chain reaction (PCR). Antibiotic resistance profile was determined by the measurement of the MIC of 14 antibiotics and by the detection of the corresponding genes using microarray and PCR approaches. Genetic diversity was determined by repetitive palindromic PCR (rep-PCR) revealing a heterogenous population. Half of the *E. coli* isolates possessing virulence factors could not be assigned to any of the 19 serotypes tested, but contained toxins and adhesins similarly to the sero-typable *E. coli* isolates. The most prevalent *E. coli* serotypes found were K88ac (18%), O139:K82 (6%), O141:K85ac (5%), O108:K`V189` (5%), O119:K`V113` (3%) and O157:K`V17` (2%). The combination of toxins EAST-1, STb and LT-I and adhesin F4 characterizing ETEC was the most frequent. The shigatoxin Stx2e (STEC) and intimin Eae (EPEC) were also detected, but less frequently. Seventy percent of the isolates were resistant to at least one antibiotic and 29%

were resistant to more than 3 antibiotics. Isolates exhibited resistance to tetracycline (50%) associated to resistance genes *tet*(A), *tet*(B) and *tet*(C), sulfamethoxazole (49%) [*sul*1, *sul*2 and *sul*3], trimethoprim (34%) [*dfr*], nalidixic acid (29%), ampicillin (26%) [bla_{TEM-1}], gentamicin (17%) [*aac(3)-IIc*, *aac(3)-IVa* and *aac(3)-VIa*], chloramphenicol (17%) [*catAI* and *catAIII*], and ciprofloxacin (8%) [mutations in GyrA (S83L) and ParC (S80I)]. All isolates were susceptible to 3rd generation cephalosporins, carbapenems, colistin and tigecycline. Pathogenic *E. coli* isolates from pigs in Switzerland could frequently not be assigned to a known serotype even if they contained diarrhea-causing virulence factors. They also harbor resistance mechanisms conferring resistance to antibiotics which are commonly used in pig husbandry, except for colistin. A careful identification of the causative agent and antibiotic susceptibility testing is highly recommended for targeted therapy and prudent use of antibiotics.

2.2.3 Novel β-Lactamase bla_{ARL} in *Staphylococcus arlettae*

Publication: Andreis SN, Perreten V, Schwendener S. 2017. mSphere **2**:e00117-17, *doi:* 10.1128/mSphere.00117-17.

Collaborators: N/A

Abstract: Whole-genome sequencing of penicillin-resistant *Staphylococcus arlettae* strain SAN1670 from bovine mastitis milk revealed a novel β-lactamase operon consisting of the β-lactamase-encoding gene bla_{ARL}, the antirepressor-encoding gene $blaR1_{ARL}$, and the repressor-encoding gene $blaI_{ARL}$. The functionality of bla_{ARL} was demonstrated by gene expression in *Staphylococcus aureus*. The bla_{ARL} operon was chromosomally located in SAN1670 and present in 10 additional unrelated strains, suggesting intrinsic penicillin resistance in *S. arlettae*. Furthermore, a GenBank search revealed more unique potential β-lactamases in *Staphylococcus* species.

2.2.4 New macrolide-lincosamide-streptogramin B resistance gene *erm*(48) on the novel plasmid pJW2311 in *Staphylococcus xylosus*

Publication: Wipf JRK, Riley MC, Kania SA, Bemis DA, Andreis S, Schwendener S, Perreten V. 2017. Antimicrob Agents Chemother **61**, *doi:* 10.1128/AAC.00066-17

Collaborators: Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee, USA

Abstract: Whole-genome sequencing of *Staphylococcus xylosus* strain JW2311 from bovine mastitis milk identified the novel 49.3-kb macrolide-lincosamide-streptogramin B (MLS_B) resistance plasmid pJW2311. It contained the macrolide resistance gene *mph*(C), the macrolide-streptogramin B resistance gene *msr*(A), and the new MLS_B resistance gene *erm*(48) and could be transformed into *Staphylococcus aureus* by electroporation. Functionality of *erm*(48) was demonstrated by cloning and expression in *S. aureus*.

Publication: Wipf JRK, Riley MC, Kania SA, Bemis DA, Andreis S, Schwendener S, Perreten V (2017) New macrolide-lincosamide-streptogramin B resistance gene *erm*(48) on the novel plasmid pJW2311 in *Staphylococcus xylosus*. Antimicrob. Agents Chemother. **61**, pii: e00066-17.

2.2.5 Plasmids carrying *bla*$_{CMY-2/4}$ in *Escherichia coli* from poultry, poultry meat, and humans belong to a novel IncK subgroup designated IncK2.

Publication: Seiffert SN, Carattoli A, Schwendener S, Collaud A, Endimiani A, Perreten V. 2017. Front Microbiol **8**:407, *doi:* 10.3389/fmicb.2017.00407

Collaborators: Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland; Institute for Infectious Diseases, University of Bern, Switzerland; Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanita, Rome, Italy

Abstract: The *bla*$_{CMY-2/4}$-carrying IncB/O/K-like plasmids of seven *Escherichia coli* strains from poultry, poultry meat and human urine samples were examined using comparative analysis of whole plasmid sequences. The incompatibility group was determined by analysis of the *inc*RNAI region and conjugation assays with strains containing the IncK and IncB/O reference plasmids. Strains were additionally characterized using MLST and MIC determination. The complete DNA sequences of all plasmids showed an average nucleotide identity of 91.3%. Plasmids were detected in *E. coli* sequence type (ST) 131, ST38, ST420, ST1431, ST1564 and belonged to a new plasmid variant (IncK2) within the IncK and IncB/O groups. Notably, one *E. coli* from poultry meat and one from human contained the same plasmid. The presence of a common recently recognized IncK2 plasmid in diverse *E. coli* from human urine isolates and poultry meat production suggests that the IncK2 plasmids originated from a common progenitor and have the capability to spread to genetically diverse *E. coli* in different reservoirs. This discovery is alarming and stresses the need of rapidly introducing strict hygiene measures throughout the food chain, limiting the spread of such plasmids in the human settings.

2.2.6 Novel methicillin resistance gene *mecD* in clinical *Macrococcus caseolyticus* strains from bovine and canine sources

Publication: Schwendener S, Cotting K, Perreten V. 2017. Sci Rep **7**:43797, *doi:* 10.1038/srep43797

Collaborators: N/A

Abstract: Methicillin-resistant *Macrococcus caseolyticus* strains from bovine and canine origins were found to carry a novel *mecD* gene conferring resistance to all classes of β-lactams including anti-MRSA cephalosporins. Association of β-lactam resistance with *mecD* was demonstrated by gene expression in *S. aureus* and deletion of the *mecD*-containing island in *M. caseolyticus*. The *mecD* gene was located either on an 18,134-bp *M. caseolyticus* resistance island (McRI$_{mecD}$-1) or a 16,188-bp McRI$_{mecD}$-2. Both islands were integrated at the 3' end of the *rpsI* gene, carried the *mecD* operon (*mecD-mecR1*$_{m}$*-mecI*$_{m}$), and genes for an integrase of the tyrosine recombinase family and a putative virulence-associated protein (*virE*). Apart from the *mecD* operon, that shared 66 % overall nucleotide identity with the *mecB* operon, McRI$_{mecD}$ islands were unrelated to any *mecB*-carrying elements or staphylococcal cassette chromosome *mec*. Only McRI$_{mecD}$-1 that is delimitated at both ends by direct repeats was capable of circular excision. The recombined excision pattern suggests site-specific activity of the integrase and allowed identification of a putative core attachment site. Detection of *rpsI*-associated integrases

in *Bacillus* and *S. aureus* reveals a potential for broad-host range dissemination of the novel methicillin resistance gene *mecD*.

2.2.7 MRSA USA300, USA300-LV and ST5-IV in pigs, Cuba

Publication: Baez M, Collaud A, Espinosa I, Perreten V. 2017. Int J Antimicrob Agents **49**:259-261, *doi:* 10.1016/j.ijantimicag.2016.12.001

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

Abstract: MRSA belonging to clonal lineages circulating in human medical settings were isolated from the nose of pigs in different provinces from Cuba. PFGE identified three major MRSA clonal clusters I, II and III. All the MRSA gathering into PFGE cluster I contained the genetic properties specific to USA300 [all ST8 except one ST173 (a single *yqil* allele variant), SCC*mec*IVa, *dru* dt9g, PVL+, ACME I+] and shared the same PFGE profile than the USA300 control strain (Fig. 1). They belonged to *spa* type t024 and also contained the *sek* and *seq* enterotoxin genes. The strains were resistant to ß-lactams (*mecA*, *blaZ*), macrolides [*msrA*, *mph*(C)], aminoglycosides [*aph(3')-III*] and fluoroquinolones [GrlA(S80-Y) and GyrA(S84-L)] (Fig. 1). MRSA clones of PFGE cluster II were characteristics to USA300-LV (ST8, *spa* t008, SCC*mec*IVc, *dru* dt7j, PVL+ and ACME-). These clones were only resistant to ß-lactams antibiotics (*mecA*, *blaZ*) and only one single strain (Cuba 7) contained the enterotoxin genes *seq* and *sek*, which are known to be either present or absent in the USA300-LV strains causing infections. The third PFGE cluster (cluster III) contained clones belonging to ST5, *spa* t010, SCC*mec* IVc, *dru* dt10a. They were all PVL- and ACME-, contained the enterotoxin genes *sea* (N315), *seb, sel, selm, seln, selo, agc,* and *selu*, and were resistant to ß-lactams (*mecA*, *blaZ*) as well as to the aminogycosides gentamicin and kanamycin (*aac(6')-Ie-aph(2'')-Ia*). This study revealed the pig husbandry of Cuba as an unsuspected reservoir of epidemic MRSA strains including those of the USA300 lineage, which have also been identified as primary causes of MRSA infections in humans in Cuba.

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

In ZOBA encompasses the three subdivisions (i) ‘Diagnostic Services and Epizootic Surveillance’, (ii) ‘Reference Laboratories and Resistance Monitoring’ and (iii) ‘Diagnostic Test Validation and Development’. The subdivisons ‘Diagnostic Services and Epizootic Surveillance’ and ‘Reference Laboratories and Resistance Monitoring’ analysed a total of 15451 and 7231 samples, respectively.

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	3288
	Necropsy material, abortion and feaces	2378
	Molecular diagnostics (PCR incl. qPCR)	1461
	Bovine mastitis	3237
	Serology	3072
	Species identification	1016
	Antibiograms for diagnostics	999
Reference Laboratories and Resistance Monitoring	Antimicrobial resistance monitoring (detection)	5214
	Antimicrobial resistance monitoring (MIC*)	1448
	Reference laboratories	569

* 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

ST Serotyping (* in human reference laboratory)

3.1.1 Highly infectious epizootics

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Contagious Bovine Pleuropneumonia	*Mycoplasma mycoides* subsp. *mycoides*	Cult	**Cattle**	0	0	0	0
		PCR		0	0	0	0
		ELISA		0	0	0	0

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**	43	34	8	1
		Cult		43	40	1	2
	Bacillus anthracis	Micr	**Swine**	0	0	0	0
		Cult		0	0	0	0
Brucellosis	*Brucella abortus*	Micr	**Cattle**	168	160	8	0
		RBT		1	1	0	0
		ELISA		634	634	0	0
		CFT		0	0	0	0
	Brucella melitensis	Micr	**Sheep/goat**	39	38	1	0
		ELISA		21	21	0	0
		CFT		0	0	0	0
		RBT		3	3	0	0
	Brucella abortus / Brucella melitensis	Micr	**Diverse**	13	13	0	0
		ELISA		1	1	0	0
		CFT		0	0	0	0
		RBT	**Diverse**	32	32	0	0
			Ibex/ chamois	2	2	0	0
	Brucella suis	Micr	**Swine**	15	15	0	0
		RBT		674	661	2	11
		ELISA		15	9	1	5
		CFT		11	10	0	1

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
	Brucella ovis	ELISA	**Sheep**	44	44	0	0

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Brucellosis	*Brucella canis*	Micr	**Dog**	3	3	0	0
		LF		16	14	0	2
Bovine Campylo-bacteriosis	*Campylobacter fetus*	Cult	**Cattle**	731	731	0	0
	subspecies *venerealis*	PCR		36	36	0	0
Sproadischer *Campylobacter* abort	*Campylobacter fetus* subspecies *fetus*	Cult	**Ruuminats**	73	73	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**	493	491	2	0
			Dog	98	51	24	23
			Horse	12	10	2	0
			Swine	71	68	3	0
	L. Autumnalis	MAT	**Dog**	97	72	7	18
			Horse	12	7	5	0
	L. Ballum	MAT	**Cattle**	50	48	2	0
			Dog	7	7	0	0
			Swine	66	66	0	0
	L. Bataviae	MAT	**Dog**	90	85	2	3
			Horse	6	6	0	0
Leptospirosis	*L.* Bratislava	MAT	**Dog**	98	70	4	24
			Horse	12	10	0	2
			Swine	69	68	1	0
	L. Canicola	MAT	**Cattle**	492	490	2	0
			Dog	98	93	4	1
			Horse	12	12	0	0
			Swine	71	71	0	0
	L. Copenhageni	MAT	**Dog**	73	46	15	12

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Leptospirosis	*L.* Grippotyphosa	MAT	**Cattle**	493	492	1	0
			Dog	98	77	11	10
			Horse	12	9	1	2
			Swine	71	69	2	0
	L. Hardjo	MAT	**Cattle**	522	457	28	37
			Dog	90	86	3	1
			Horse	6	6	0	0
	L. Icterohaemo-rrhagiae	MAT	**Cattle**	493	493	0	0
			Dog	97	93	3	1
			Horse	11	11	0	0
			Swine	71	71	0	0
	L. Pomona	MAT	**Cattle**	501	497	2	2
			Dog	98	71	11	16
			Horse	12	12	0	0
			Swine	71	71	0	0
	L. Pyrogenes	MAT	**Dog**	89	83	3	3
			Horse	11	11	0	0
	L. Sejroe	MAT	**Cattle**	532	475	48	9
			Dog	27	25	2	0
			Horse	6	6	0	0
	L. Tarasosovi	MAT	**Cattle**	84	84	0	0
			Dog	89	89	0	0
			Horse	11	10	1	0
			Swine	71	71	0	0
	Leptospira spp	PCR	**Swine**	10	9	0	1
			Cattle	40	45	0	5
			Horse	3	2	0	1
			Dog	14	10	1	3
			Beaver	22	10	1	11

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Salmonellosis		Cult/ST	**Alpaca**	1	1	0	0
			Bat	1	1	0	0
			Bear	2	2	0	0
			Beaver	1	1	0	0
			Bird	35	35	0	0
			Bison	6	6	0	0
			Cat	23	23	0	0
			Cattle	249	231	0	18
	S. Enteritidis						4
	S. Typhimurium						5
	S. Typhimurium, monophasic variant (4,12 : i : -)						8
	S. enterica subsp. *diarizonae* 61:k:1,5,7						1
			Chamois	2	2	0	0
			Chicken	7	7	0	0
			Coendu	11	11	0	0
			Deer	12	12	0	0
			Dog	62	60	0	2
	S. Albany						1
	S. Chester						1
			Duck	3	3	0	0
			Eagle	1	1	0	0
			Elephant	2	2	0	0
			Frog	1	1	0	0
			Gepard	2	2	0	0
			Giraffe	2	2	0	0
			Guinea pig	1	1	0	0
			Goose	2	2	0	0
			Gorilla	3	3	0	0
			Goat	16	16	0	0
			Hedgehoge	1	1	0	0

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Salmonellosis		Cult/ST	**Horse**	64	64	0	0
			Ibex	1	1	0	0
			Iguana	2	1	0	1
	S. Eastbourne						1
			Kangaroo	2	2	0	0
			Leopard	3	3	0	0
			Leeser mouse deer	1	1	0	0
			Lynx	1	1	0	0
			Monkey	7	7	0	0
			Mouse	3	3	0	0
			Musk ox	2	2	0	0
			Okapi	4	4	0	0
			Orang Utan	1	1	0	0
			Otter	4	4	0	0
			Penguin	2	2	0	0
			Rat	2	2	0	0
			Rhinoceros	3	3	0	0
			Rabbit	2	2	0	0
			Saurian	2	0	0	2
	S. enterica subsp. *diarizonae* 61:z52:z53*-						1
	S. Havanna						1
			Sheep	28	23	0	5
	S. enterica subsp. *diarizonae* 61:k:1,5,7						5
			Sloth	1	1	0	0

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Salmonellosis		Cult/ST	**Snake**	7	0	0	7
	S. Florida						1
	S. enterica subsp. *enterica* 6,7:k:-*						1
	S. enterica subsp. *arizonae* 44:z4,z24,-*						1
	S. enterica subsp. *arizonae* 41:z4,z23:-*						1
	S. enterica subsp. *arizonae* 21:-:-*						1
	S. enterica subsp. *arizonae* 56:k:z4,z23:-						1
	S. enterica subsp. *diarizonae* 65:z10:e,n,x,z15						1
			Swine	92	88	0	4
	S. Enteritidis						2
	S. Bredeney						1
	S. Typhimurium, monophasic variant *(4,12 : i : -)*						1
			Tortoise	6	6	0	0
			Wild boar	1	1	0	0
			Wisent	1	1	0	0
Contagious equine metritis	*Taylorella equigenitalis*	Cult	**Horse**	79	79	0	0
Enzootic pneumonia in swine	*Mycoplasma hyopneumoniae*	PCR Lung	**Swine**	105	97	0	8
		PCR Nasal swab		62	56	0	6
		PCR Project		848	725	0	123
		ELISA		456	393	13	50
		ELISA Project		624	543	24	57

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Swine actinobacillosis	*Actinobacillus pleuropneumoniae*	Cult/PCR	**Swine**	145	97	0	48
	I BD +II CA, Serotyp 7,12						36
	I BD+II CA+III CA+BD, Serotyp 2						5
	I BD + II CA, Serotyp 2						1
	II CA+IIICA+BD, Serotyp 3						6
		ELISA ApxIV		75	49	7	19

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		Cult	**Dog**	52	48	0	0
	C. jejuni						4
			Cat	19	19	0	0
			Cattle	34	22	0	12
	C. jejuni						7
* not thermotolerant	*C. fetus**						5
			Monkey	6	6	0	0
			Diverse	24	22	0	0
	C. jejuni						2
Listeriosis	*Listeria monocytogenes*	Cult	**Ruminants**	13	10	0	3
Yersiniosis	*Yersinia* spp.	Cult	**Swine**	1	0	0	1
			Fish	3	0	0	3
Caseous lymphadenitis in sheep and goats	*Corynebacterium pseudotuberculosis (ovis)*	Cult	**Goat**	10	5	0	5
			Sheep	3	0	0	3
			Diverse	5	0	0	5

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

Epizootic	Agent	Method	Host	Total	Negative	Suspicious	Positive
Enzootic abortion in ewes (chlamydiosis)	*Chlamydia abortus*	Micr	**Sheep**	9	9	0	0
		ELISA		6	5	0	1
		PCR		3	3	0	0
		Micr	**Goat**	12	11	1	0
		ELISA		2	2	0	0
		PCR		5	4	0	1
		Micr	**Cattle**	64	60	4	0
		ELISA		14	6	1	7
		PCR		30	30	0	0
Psittacosis	*Chlamydia psittaci*	PCR	**Bird**	9	9	0	0
Tularaemia	*Francisella tularensis*	Cult	**Monkey**	1	1	0	0
		Cult	**Rat**	3	3	0	0
Blackleg	*Clostridium chauvoei*	IF	**Ruminant**	30	22	3	5
		Cult		30	24	1	5
Coxiellosis	*Coxiella burnetii*	Micr	**Cattle**	166	158	8	0
		ELISA		14	11	1	2
		PCR		33	18	0	15
		Micr	**Sheep**	16	16	0	0
		ELISA		20	20	0	0
		PCR		2	2	0	0
		Micr	**Goat**	18	17	1	0
		ELISA		2	2	0	0
		PCR		7	3	0	4

3.2 Annual Antimicrobial Resistance Monitoring

Program concerning 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. Caecal samples from pigs and veal calves were collected at slaughter and cultured for *E. coli*, *Enterococcus* spp., *Campylobacter* spp., extended spectrum beta-lactamases (ESBLs), carbapenemases producing *E. coli* as well as colistin-resistant *E. coli*. Moreover, fresh meat thereof from retail was analysed for ESBLs and carbapenemases producing *E. coli*, colistin-resistant *E. coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Isolated strains and all *Salmonella* Typhimurium including its monophasic variant and *Salmonella* Enteritidis 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 Swiss antibiotic resistance report, Federal Office of Public Health (FOPH) and 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 2017 antimicrobial resistance monitoring are displayed in tables 6a to 6h.

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

Matrix	Number of analyses
pig meat samples	301
beef samples	299
Total	**600**

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

Matrix	Number of analyses
pig caecal samples	296
pig meat samples	301
veal calves caecal samples	304
beef meat samples	299
Total	**1200**

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

Matrix	Number of analyses
pig caecal samples	296
pig meat samples	302
veal calves caecal samples	304
beef meat samples	299
Total	**1201**

Table 6d: Number of analyses on colistin-resistant *E. coli*

Matrix	Number of analyses
pig caecal samples	296
pig meat samples	302
veal calves caecal samples	304
beef meat samples	299
Total	**1201**

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

Matrix	Number of analyses
pig caecal samples	216
veal calves caecal samples	204
Total	**420**

Table 6f: Number of analyses on *Enterococcus* spp.

Matrix	Number of analyses
veal calves caecal samples	296
Total	**296**

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

Matrix	Number of analyses
pig caecal samples	296
Total	**296**

Table 6h: Number of analyses on *Salmonella* spp.

Salmonella serovar	Number of analyses
S. Enteritidis	28
S. Typhimurium	58
S. Typhimurium, monophasic variant	31
Total	**117**

3.3 Reference Activity for Epizootics (Notifiable Animal Diseases)

3.3.1 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	Host	Total	Negative	Suspicious	Positive
Anthrax	Micr	**cattle**	0	0	0	0
	Cult		0	0	0	0
Bovine brucellosis	ELISA	**cattle**	14	7	2	5
	RBT		13	12	0	1
	CFT		12	9	2	0
Caprine and ovine Brucellosis	ELISA	**sheep/ goat**	1	1	0	0
	RBT		1	0	0	1
	CFT		1	1	0	0
Porcine brucellosis	ELISA	**swine**	17	10	0	7
	RBT		17	10	1	0
	CFT		17	15	2	0
Canine brucellosis	LF	**dog**	2	0	0	2
	Micr		0	0	0	0
	Cult		0	0	0	0
	Direct PCR		2	0	0	59
Ovine epididymitis (*Brucella ovis*)	ELISA	**sheep**	0	0	0	0
Swine actinobacillosis	ELISA ApxIV	**swine**	0	0	0	0
	Cult		62	3	0	59
	PCR		59	0	0	59
Contagious equine Metritis	Cult	**horse**	0	0	0	0
	PCR		0	0	0	0
Blackleg	IF	**cattle**	3	3	0	0
	Cult		4	4	0	0
	PCR		0	0	0	0

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

Epizootic	Method	Host	Total	Negative	Suspicious	Positive
Campylobacteriosis	ID	**chicken**	2	0	0	2
		diverse	3	0	0	3
Enzootic pneumonia in swine	PCR Lung	**swine**	0	0	0	0
Yersiniosis	ID	**fish**	0	0	0	0
	ID	**cattle**	1	0	0	1
Multidrug resistance	ID, MIC	**diverse**	48	0	0	48
Identification	ID	**diverse**	10	0	0	10

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

Table 8: Number of *Salmonella* spp. isolates for serotyping received from other laboratories

Serovar	Host	Number
S. Agona	Dog	1
S. Albany	Dog	1
	Turkey	6
S. Bukavu*	Lizard	1
S. Carmel*	Laying hen	1
S. Derby	Swine	4
S. Dublin	Cattle	2
	Cat	1
S. Enteritidis	Cattle	8
	Chicken	9
	Laying hen	8
	Breeding hen	1
	Bird	1

Table 8: Number of *Salmonella* spp. isolates for serotyping received from other laboratories (continuation)

Serovar	Host	Number
S. enterica subsp. *arizonae* 41: z4,z23: -*	Snake	1
S. enterica subsp. *diarizonae* 61: -: 1,5,7	Sheep	2
S. enterica subsp. *diarizonae* 61: k: 1,5,7	Sheep	6
S. enterica subsp. *diarizonae* 38: l,v:-*	Snake	1
S. enterica subsp. *diarizonae* 50: k:z	Lizard	1
S. enterica subsp. *diarizonae* 53: k:e,n,z15	Chameleon	2
S. enterica subsp. *enterica* 16: l,v:-	Lizard	1
S. enterica subsp. *enterica* 13,23 :i:-	Broiler	2
S. enterica subsp. *enterica* - 11:h:-	Breeding hen	1
S. enterica subsp. *enterica* 11:-:e,n,x	Chicken	1
S. enterica subsp. *houtenae* 11:z4,z23:-	Lizard	1
S. enterica subsp. *houtenae* 44: z4,z23 -	Lizard	1
S. Fluntern	Chicken	3
S. Fresno*	Broiler	1
S. Havanna	Chicken	1
S. Isangi	Dog	1
S. Livingstone	Laying hen	1
S. Luke*	Broiler	1
S. Mbandaka	Chicken	9
	Laying hen	6
	Breeding hen	1
S. Meleagridis	Dog	1
S. Menston	Chicken	1
S. Monschaui	Lizard	1
S. Napoli	Cattle	1
	Laying hen	1
S. Newport	Cattle	1
	Chicken	1

Table 8: Number of *Salmonella* spp. isolates for serotyping received from other laboratories (continuation)

Serovar	Host	Number
S. Oranienburg	Chicken	2
S. Senftenberg	Chicken	2
	Laying hen	1
S. Tennessee	Broiler	1
S. Typhimurium	Cattle	44
	Chicken	4
	Laying hen	20
S. Typhimurium	Unknown	1
	Goat	1
	Dog	1
	Bird	3
	Broiler	2
	Turkey	1
	Horse	2
S. Typhimurium, monophasic variant (4,12 : i : -)	Cattle	19
	Laying hen	2
	Broiler	2
	Swine	7
	Dog	1
S. Veneziana	Breeding hen	4
S. Woumbou	Snake	1
No salmonella		2
	Total	**218**

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

Table 8: 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	39
Biovar I	apx group: I BD + II CA + III CA + BD *cps*2 gene positive	2	6
Biovar I	apx group: I CA+ II CA + BD	1,5,9,11	3
Biovar I	apx group: III CA + BD	3 variant	0
Biovar II	apx group: I BD + II CA *cps*2 gene positive	2	11
No APP	-	-	3
Total			**62**

3.4 Research Activities

3.4.1 New approaches to abortion diagnostics in ruminants

3.4.1.1 Amplicon sequencing of bacterial microbiota in abortion material from cattle

Publication: Vidal S, Kegler K, Greub G, Aeby S, Borel N, Dagleish MP, Posthaus H, Perreten V, Rodriguez-Campos S. 2017. BMC Vet Res **13**:373, *doi:* 10.1186/s12917-017-1294-y.

Collaborators: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland

Abstract: Abortions in cattle have a significant economic impact on animal husbandry and require prompt diagnosis for surveillance of epizootic infectious agents. Since most abortions are not epizootic but sporadic with often undetected etiologies, this study examined the bacterial community present in the placenta (PL, n = 32) and fetal abomasal content (AC, n = 49) in 64 cases of bovine abortion by next generation sequencing (NGS) of the 16S rRNA gene. The PL and AC from three fetuses of dams that died from non-infectious reasons were included as controls. All samples were analyzed by bacterial culture, and 17 were examined by histopathology. We observed 922 OTUs overall and 267 taxa at the genus level. No detectable bacterial DNA was present in the control samples. The microbial profiles of the PL and AC differed significantly, both in their composition (PERMANOVA), species richness and Chao-1 (Mann–Whitney test). In both organs, *Pseudomonas* was the most abundant genus. The combination of NGS and culture identified opportunistic pathogens of interest in placentas with lesions, such as *Vibrio metschnikovii*, *Streptococcus uberis*, *Lactococcus lactis* and *Escherichia*

coli. In placentas with lesions where culturing was unsuccessful, *Pseudomonas* and unidentified *Aeromonadaceae* were identified by NGS displaying high number of reads. Three cases with multiple possible etiologies and placentas presenting lesions were detected by NGS. Amplicon sequencing has the potential to uncover unknown etiological agents. These new insights on cattle abortion extend our focus to previously understudied opportunistic abortive bacteria.

3.4.1.2 Neglected zoonotic agents in cattle abortion

Publication: Vidal S, Kegler K, Posthaus H, Perreten V, Rodriguez-Campos S. 2017. Vet Res **48**:64, *doi:* 10.1186/s13567-017-0470-1

Collaborators: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland; Institute of Veterinary Pathology Vetsuisse Faculty, University of Zurich, Switzerland; Institute of Microbiology of the University Hospital Center, University of Lausanne, Switzerland; Moredun Research Institute, Scotland, UK

Abstract: Coxiella burnetii, *Chlamydia abortus* and *Leptospira* spp. are difficult to grow bacteria that play a role in bovine abortion, but their diagnosis is hampered by their obligate intracellular lifestyle (*C. burnetii, C. abortus*)or their lability (*Leptospira* spp.). Their importance is based on the contagious spread in food-producing animals, but also as zoonotic agents. In Switzerland, first-line routine bacteriological diagnostics in cattle abortions is regulated by national law and includes only basic screening by staining for *C. burnetii* due to the high costs associated with extended spectrum analysis. The aim of this study was to assess the true occurrence of these zoonotic pathogens in 249 cases of bovine abortion in Switzerland by serology (ELISA for anti-*C. burnetii* and *C. abortus* antibodies and microscopic agglutination test for anti-*Leptospira* spp. antibodies), molecular methods (real-time PCR and sequencing of PCR products of Chlamydiales-positive cases), Stamp's modification of the Ziehl-Neelsen (mod-ZN) stain and, upon availability of material, by histology and immunohistochemistry (IHC).

After seroanalysis the prevalence was 15.9% for C. burnetii, 38.5% for C. abortus and 21.4% for *Leptospira* spp. By real-time PCR 12.1% and 16.9% of the cases were positive for *C. burnetii* and *Chlamydiales*, respectively, but only 2.4% were positive for C. burnetii or *Chlamydiales* by mod-ZN stain. Sequencing of PCR products of *Chlamydiales*-positive cases revealed *C. abortus* in 10% of cases and the presence of a mix of *Chlamydiales*-related bacteria in 5.2% of cases. Pathogenic *Leptospira* spp. were detected in 5.6% of cases. Inflammatory lesions were present histologically in all available samples which were real-time PCR-positive for *Chlamydiales* and *Leptospira* spp. One of 12 real-time PCR-positive cases for *C. burnetii* was devoid of histological lesions. None of the pathogens could be detected by IHC.

Molecular detection by real-time PCR complemented by histopathological analysis is recommended to improve definitive diagnosis of bovine abortion cases and determine a more accurate prevalence of these zoonotic pathogens.

3.4.1.3 Broad-spectrum diagnostics of abortion in small ruminants

Publication: Schnydrig P, Vidal S, Brodard I, Frey C, Posthaus H, Perreten V, Rodriguez-Campos S. 2017. Schweiz Arch Tierheilkd **159**:647-656, *doi:* 10.17236/sat00136.

Collaborators: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland; Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland

Abstract: Abortion in small ruminants presents a clinical and economic problem with legal implications regarding animal health and zoonotic risk by some of the abortive pathogens. Several bacteria, fungi and parasites can cause abortion, but cost-orientated routine diagnostics only cover the most relevant epizootic agents. To cover a broad-range of common as well as underdiagnosed abortifacients, we studied 41 ovine and 36 caprine abortions by Stamp's modification of the Ziehl-Neelsen stain, culture for classical and opportunistic abortive agents, real-time PCR for *C. burnetii, C. abortus,* pathogenic *Leptospira* spp., *Toxoplasma gondii* and *Neospora caninum*. When the dam's serum was available detection of antibodies against *B. melitensis, C. burnetii, C. abortus* and *Leptospira* spp. was performed. In 37 cases sufficient placental tissue was available for pathological and histopathological examination. From the 77 cases 11 (14.3%) were positive by staining whereas real-time PCR detected *C. burnetii* and *C. abortus* in 49.3% and 32.5% of the cases. Antibodies against *C. abortus* and *Leptospira* spp. (33.3 and 26.7%) were detected. In 23.4% a bacterial culturable pathogen was isolated. Fungal abortion was confirmed in 1.3% of cases. A single abortive agent was identified in 44.2% of the cases and in 31.2% multiple possible abortifacients were present. Our study shows that the highest clarification rate can only be achieved by a combination of methods and evidences the role that multi-infections play as cause of abortion.

3.4.2 Diagnostics of animal leptospirosis

3.4.2.1 Clinical, serological and echocardiographic examination of healthy field dogs before and after vaccination with a commercial tetravalent leptospirosis vaccine

Publication: Spiri AM, Rodriguez-Campos S, Matos JM, Glaus TM, Riond B, Reusch CE, Hofmann-Lehmann R, Willi B. 2017. BMC Vet Res **13**:138, *doi:* 10.1186/s12917-017-1056-x.

Collaborators: Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland; Center for Clinical Studies, Vetsuisse Faculty, University of Zurich, Switzerland; Clinic for Small Animal Internal Medicine, University of Zurich, Switzerland

Abstract: Leptospirosis is a re-emerging bacterial zoonosis caused by spirochetes of the genus *Leptospira*. Severe disease has been reported in dogs in Europe despite vaccination with bivalent Leptospira vaccines. Recently, a tetravalent canine *Leptospira* vaccine (Nobivac® L4) was licenced in Europe. The goal of this study was to investigate clinical signs, microscopic agglutination test (MAT) titres, haematology, blood biochemistry, cardiac (c) Troponin I levels and echocardiography before and after vaccination with this tetravalent vaccine. Forty-eight healthy dogs were prospectively enrolled and vaccinated twice, 3–4 weeks apart (T0 and T1). Before vaccination (T0) and 16–31 days after the second vaccination (T2), MAT (n = 48), haematology (n = 48), blood biochemistry (n = 36) and cTroponin I measurements (n = 29)

were performed, and MAT was repeated 347–413 days after the second vaccination (T3, n = 44). Echocardiography was performed before the first and second vaccination (T0 and T1, n =24).

Mild and transient clinical signs within 5 days following the first and second vaccination occurred in 23% and 10% of the dogs, respectively. Before the first vaccination (T0), all dogs showed negative MAT titres for the tested serovars except for Canicola (50% with titres 100–400). At T2, positive MAT titres to the serovars Canicola (100%), Australis (89%), Grippotyphosa (86%), Bratislava (60%), Autumnalis (58%), Copenhageni (42%), Pomona (12%), Pyrogenes (8%) and Icterohaemorrhagiae (2%) were found. Median to high titres (≥ 400) were most common to the serovar Canicola (92%) and less common to the serovars Australis (41%), Grippotyphosa (21%), Bratislava (12%), Autumnalis (4%), Pyrogenes (4%) and Pomona (2%). At T3, positive MAT titres (titre range: 100– 400) were found in 2–18% of the dogs to serovars of the vaccine serogroups and in 2–18% of the dogs to the non-vaccine serovars Pomona, Autumnalis, Pyrogenes and Ballum. Haematology, blood biochemistry, cTroponin I levels and echocardiography results did not change significantly following vaccination. Clinical signs following vaccination with Nobivac® L4 were transient and mild in all cases. Seroconversion differed considerably among individual dogs and among the vaccine serogroups.

3.4.2.2 Canine leptospirosis in Switzerland – Seroprevalence, risk factors and urinary shedding of pathogenic leptospires

Publication: Delaude A, Rodriguez-Campos S, Dreyfus A, Counotte MJ, Francey T, Schweighauser A, Lettry S, Schuller S. 2017. Prev Vet Med **141**:48-60, *doi:* 10.1016/j.prevetmed.2017.04.008

Collaborators: Small Animal Clinic, Vetsuisse Faculty, University of Bern, Switzerland; Section of Epidemiology, Vetsuisse Faculty, University of Zürich, Switzerland

Abstract: Leptospirosis is an important worldwide zoonosis. While human leptospirosis remains rare in Switzerland, the incidence of canine leptospirosis is unusually high compared to other European countries. The aims of this cross-sectional study were to determine the exposure of asymtomatic dogs to pathogenic *Leptospira* in Switzerland, to characterise risk factors associated with seropositivity and to determine the prevalence of urinary shedding. Sampling was stratified to cover the whole of Switzerland. Sera were tested by microscopic agglutination test for antibodies against a panel of 12 serovars. Urine was tested for pathogenic *Leptospira* using a LipL32 real-time PCR. Of 377 sera, 55.7% (95%CI 51.2–60.7) showed a reciprocal MAT titre of ≥1:40 and 24.9% (95%CI 20.7–29.4) of ≥1:100 to at least one serovar. Seropositivity (MAT ≥1:100) was most common to serovars Australis (14.9%; 95% CI 11.4–18.6) and Bratislava (8.8%; 95%CI 6.1–11.7), followed by Copenhageni (6.1%; 95%CI 3.7-8.5), Canicola (5%; 95%CI 2.9–7.4), Grippotyphosa (4.5%; 95%CI 2.7–6.9), Pomona (4%; 95%CI 2.1–6.1), Autumnalis (2.7%; 95%CI 1.3–4.2) and Icterohaemorrhagiae (1.6%; 95%CI 0.5-2.9). In unvaccinated dogs (n = 84) the prevalence of a MAT titre ≥100 was 17.9% (95%CI 10.7–26.2), with a similar distribution of reactive serovars. Variables associated with

seropositivity (≥1:40) to any serovar included age (OR 1.29/year; 95%CI: 1.1–1.5) and bioregion with higher risks in the regions Northern Alps (OR 14.5; 95%CI 2.2–292.7), Central Plateau (OR 12.3; 95%CI 2.0–244.1) and Jura (OR 11.2; 95%CI 1.7–226.7) compared to Southern Central Alps. Dogs living with horses were significantly more likely to have antibodies to serovar Bratislava (OR 4.68;95%CI 1.2–17.2). Hunting was a significant risk factor for seropositivity to serovar Grippotyphosa (OR 8.03; 95%CI 1.6–30.8). Urine qPCR positivity was uncommon (1/408 dogs; 0.2%; 95% CI0–0.7). These results demonstrate that dogs in Switzerland are commonly exposed to pathogenic *Leptospira*; however, the risk of dogs contributing to the spread of *Leptospira* in the environment appears low.

3.4.2.3 Comparison of „patient-side“ tests for canine leptospirosis with the gold standard

Publication: Gloor CI, Schweighauser A, Francey T, Rodriguez-Campos S, Vidondo B, Bigler B, Schuller S. 2017. J Small Anim Pract **58**:154-161, *doi:* 10.1111/jsap.12628

Collaborators: Small Animal Clinic, Vetsuisse Faculty, University of Bern, Switzerland

Abstract: To determine the diagnostic performance of two patient-side tests (RDT-1: Test-it™ and RDT-2 Witness®Lepto) in the early diagnosis of canine leptospirosis. Retrospective study of 108 dogs with leptospirosis and 53 controls. Leptospirosis was diagnosed based on compatible clinical and clinicopathologic signs and either a single microscopic agglutination test titre_ >800 (n=49), seroconversion (n=53), positive urine real time PCR (RT-PCR) (n=1), evidence of spirochaetes in silver-stained tissues (n=1) or a combination of these (n=4). Leptospirosis was excluded in dogs with a convincing alternative diagnosis and single microscopic agglutination testing titres _<200 (n=46) or lack of seroconversion (n=7). Indices of diagnostic accuracy of the rapid diagnostic tests were calculated by comparing admission rapid diagnostic test results to the final disease status. Rapid diagnostic test-1 was performed in 118 dogs, rapid diagnostic test-2 in 69 dogs and both tests in 26 dogs. Weak positive results occurred frequently representing 22·6% (rapid diagnostic test1) and 32·3% (rapid diagnostic test-2) of all positive tests in dogs with leptospirosis. If weak positive rapid diagnostic tests were considered positive, rapid diagnostic test-1 and rapid diagnostic test-2 had sensitivities of 82 and 76%, specificities of 91 and 100%, positive predictive values of 94% and 100% and negative predictive values of 73% and 74%, respectively. There were some technical problems with rapid diagnostic test-1. The diagnostic performance of the rapid diagnostic tests is similar to that reported for the microscopic agglutination test. Both can support a diagnosis of leptospirosis with high specificity but leptospirosis cannot be excluded based on a negative admission test result. Both RDTs are useful in conjunction with other confirmatory tests.

3.4.3 Diagnostics of leptospirosis in Uganda

Collaborative study with scientists from the Section of Epidemiology Vetsuisse Faculty Zurich (Switzerland) and the College of Veterinary Medicine, Animal Resources & Biosecurity of the Makerere University (Uganda)

Our implication in One Health activities led by the Section of Epidemiology of the Vetsuisse Faculty Zurich made it possible to train Ugandan laboratory staff and to transfer technology for molecular and serological diagnostics of leptospirosis to the Makerere University.

3.4.3.1 Case of clinical canine leptospirosis in Uganda

Publication: Alinaitwe L, Kakooza S, Eneku W, Dreyfus A, Rodriguez-Campos S. Vet Rec Case Reports. 2017; **5**: e000484, *doi:* 10.1136/vetreccr-2017-000484

Collaborators: Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Switzerland; College of Veterinary Medicine, Animal Resources & Biosecurity, Makerere University, Uganda

Abstract: For the first time a case of clinical leptospirosis in a dog in Uganda could be confirmed using real-time PCR. A five-year-old entire male German shepherd dog from a kennel of 25 guard dogs was admitted to the animal clinic and presented hypothermia, lethargy and jaundice of the mucous membranes. The body temperature remained low during the three days post admission until death. The postmortem examination and histological findings led to suspicion of acute leptospirosis. The diagnosis was confirmed by demonstration of pathogenic *Leptospira* spp. DNA in homogenates of the kidney, liver and lungs by real-time PCR. This case highlights that accurate diagnostic methods are needed to clarify if clinical leptospirosis is to date underestimated in Uganda and if it has an impact on public health. Awareness should be raised among veterinarians to consider leptospirosis more often as a differential diagnosis because of the non-specific signs observed in the presented case.

3.4.3.2 Cross-sectional serological survey for *Leptospira* spp. in beef and dairy cattle in two districts in Uganda.

Publication: Dreyfus A, Odoch T, Alinaitwe L, Rodriguez-Campos S, Tsegay A, Jaquier V, Kankya C. 2017. Int J Environ Res Public Health **14**, *doi:* 10.3390/ijerph14111421

Collaborators: Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Switzerland; College of Veterinary Medicine, Animal Resources & Biosecurity, Makerere University, Uganda

Abstract: In a further study the seroprevalence of *Leptospira* spp.antibodies in cattle in Uganda was determined. The aim of this study was to estimate the seroprevalence of antibodies against *L. interrogans* Icterohaemorrhagiae, Pomona, *L. kirschneri* Butembo, Grippotyphosa, *L. borgpetersenii* Nigeria, Hardjo, Wolfii, and Kenya and an overall seroprevalence in cattle from Kole and Mbale districts. Two hundred-seventy five bovine sera from 130 small holder farms from Kole (n = 159) and Mbale (n = 116), collected between January and July 2015, were tested for antibodies against eight Leptospira strains by Microscopic Agglutination Test. A titer of ≥100 was considered seropositive, indicating past exposure. Overall, the seroprevalence was 19.27% (95% CI 14.9–24.5%). Pomona seroprevalence was highest with 9.45% (6.4–13.7%), followed by Kenya 5.09% (2.9–8.6%), Nigeria 4.00% (2.1–7.2%),Wolfii 3.27% (1.6–6.3%), Butembo 1.86% (0.7–4.4%), Hardjo 1.45% (0.5–3.9%), and Icterohaemorragiae and Grippotyphosa with less than 1% positive. Seroprevalence did not differ between districts and

gender ($p \geq 0.05$). Seven animals had titers ≥400. Cross-reactions or exposure to more than one serovar was measured in 43% of serum samples. Seroprevalence of 19% implies exposure of cattle to leptospires.

3.4.4 *Macrococcus canis* sp. nov., a skin bacterium associated with infections in dogs

Publication: Gobeli Brawand S, Cotting K, Gomez-Sanz E, Collaud A, Thomann A, Brodard I, Rodriguez-Campos S, Strauss C, Perreten V. 2017. Int J Syst Evol Microbiol **67**:621-626, *doi:* 10.1099/ijsem.0.001673

Collaborators: N/A

Abstract: Gram-stain-positive cocci were isolated from miscellaneous sites of the skin of healthy dogs as well as from infection sites in dogs. The closest relative by sequencing of the 16S rRNA gene was *Macrococcus caseolyticus* with 99.7% sequence identity, but compared with *M. caseolyticus*, the novel strains shared only 90.8 to 93.5% DNA sequence identity with *cpn60, dnaJ, rpoB* and *sodA* partial genes, respectively. The novel strains also exhibited differential phenotypic characteristics from *M. caseolyticus*, and the majority displayed a visible haemolysis on sheep blood agar, while *M. caseolyticus* did not have any haemolytic activity. They generated different matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) MS spectral profiles compared with the other species of the genus *Macrococcus*. Furthermore, strain KM 45013T shared only 53.7% DNA-DNA relatedness with the type strain of *M. caseolyticus*, confirming that they do not belong to the same species. The DNA G+C content of strain KM 45013T was 36.9 mol%. The most abundant fatty acids were C14 : 0, C18 : 3ω6c (6, 9, 12) and C16 : 0 n alcohol. MK-6 was the menaquinone type of KM 45013T. Cell-wall structure analysis revealed that the peptidoglycan type was A3α l-Lys-Gly2-l-Ser. Based on genotypic and chemotaxonomic characteristics, we propose to classify these strains within a novel species of the genus *Macrococcus* for which the name *Macrococcus canis* sp. nov. is proposed. The type strain is KM 45013T (=DSM 101690T=CCOS 969T=CCUG 68920T).

3.4.5 *Salmonella enterica* subsp. *diarizonae* serotype 61:k:1,5,(7) associated with chronic proliferative rhinitis and high nasal colonization rates in a flock of Texel sheep in Switzerland

Publication: Stokar-Regenscheit N, Overesch G, Giezendanner R, Roos S, Gurtner C. 2017. Prev Vet Med 145:78-82, doi: 10.1016/j.prevetmed.2017.07.003

Collaborators: Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland; Swiss Health Service for Small Ruminants, Herzogenbuchsee, Switzerland

Abstract: Salmonella (S.) enterica subspecies *diarizonae* (IIIb) serovar 61:(k):1,5,(7) (S. IIIb 61:(k):1,5,(7)) is considered to be host adapted to sheep and is found regularly in feces of healthy carriers and of sheep with salmonellosis. A few cases of chronic proliferative rhinitis (CPR) in sheep have been described as a new disease in association with S. IIIb 61:k:1,5,(7) in the USA, in Spain and now for the first time in Switzerland. Three animals of a flock of Texel sheep suffering from chronic nasal discharge and dyspnea with subsequent death were

necropsied. The pathological lesions are consistent with a severe proliferation of the nasal mucosae of the turbinates in association with severe chronic inflammation. S. IIIb 61:(k):1,5,(7) was isolated from the lesions by direct bacteriological culture and the presence of Salmonella sp.spp. was confirmed by immunohistochemistry. The affected flock was systematically tested after the first occurrence of the disease. Clinical examination of the flock revealed approx. 20 % of the adult sheep to show nasal discharge, approx. 5 % having severe dyspnea and approx. 5 % having chronic intermittent diarrhea. Lambs (n=28) showed no clinical signs at all. High positivity of nasal mucosa (45.86 %), but low prevalence in feces (5.96 %) for S. IIIb 61:k:1,5,(7) was found. The results lead to the assumption of a direct animal to animal transmission by nasal discharge followed by a chronic disease leading to death after several months to years. Animals tested positive for S. IIIb 61:k:1,5,(7) were all >1 year old. CPR represents a chronic disease in adult sheep posing a risk for spreading S. IIIb 61:k:1,5,(7) between flocks and with a zoonotic potential.

3.4.6 A quantitative real-time PCR approach for assessing *Campylobacter jejuni* and *Campylobacter coli* colonization in broiler herds

Publication: Haas K, Overesch G, Kuhnert P. 2017. J Food Prot **80**:604-608, *doi:* 10.4315/0362-028X.JFP-16-395

Collaborators: N/A

Abstract: Human campylobacteriosis is of major public health concern in developed countries with Campylobacter (C.) jejuni and C. coli from poultry recognized as the main source of human infection. Identification of Campylobacter-positive broiler herds before slaughter is essential for implementing measures to avoid carry-over of pathogens via the slaughter process into the food chain. However, appropriate methods and their validation to test poultry flocks ante mortem are missing for Campylobacter. A quantitative real-time PCR (qPCR) that allows simultaneous detection and, quantification and differentiation of C. jejuni and C. coli was adapted and optimized and adapted to be applied on boot socks. The adjusted qPCR serves an easy, sensitive and quantitative method for Campylobacter detection in poultry flocks ante mortem by analysis of boot socks. An adequate correlation was found between qPCR and culture, as well as between boot socks and cecal samples, regarded as gold standard. Therefore, boot sock sampling followed by qPCR analysis provides a reliable and simple means for assessing campylobacter load within a flock prior to slaughter. The method allows categorization of broiler herds into negative, low, moderate or high Campylobacter colonization. Based on the results of this new qPCR, risk assessment models like such as evaluating the possible effect of sorting flocks before slaughter, can be easily implemented. Similarly, targeted identification of highly colonized flocks for improvement of biosecurity measures on the farm level will become feasible presenting an opportunity to increase food safety.

3.4.6 Persistence of *Mycoplasma hyopneumoniae* sequence types in spite of a control program for enzootic pneumonia in pigs

Publication: Overesch G, Kuhnert P. 2017. Prev Vet Med **145**:67-72, *doi:* 10.1016/j.prevetmed.2017.06.007.

Collaborators: N/A

Abstract: Enzootic pneumonia (EP) in pigs caused by *Mycoplasma (M.) hyopneumoniae* has successfully been combatted in Switzerland. A control program was fully implemented in 2004 which is based on total depopulation strategies of affected fattening farms as well as partial depopulation on breeding farms. Thereby, the number of outbreaks has dropped drastically from more than 200 in 2003 to two cases in 2013. Currently monitoring is done based on clinical observation and subsequent diagnostic of coughing pigs. Moreover, in case of more than 10% gross pathological lesions per slaughter batch laboratory confirmation for EP is compulsory. Despite these strict measures it was not possible to eradicate eliminate enzootic pneumonia. *M. hyopneumoniae* from Swiss pig production. In fact, during the last few years the number of EP cases has slightly increased. Therefore, genotyping of the involved *M. hyopneumoniae* strains was conducted in order to elucidate possible sources and routes of infection. All available and typeable samples from concurrent outbreaks during the period 2014-2016 were investigated by extended multilocus sequence typing (MLST). A total of 16 samples, including eight samples from 2014, five samples from 2015 and three samples from 2016 could be included in the study. MLST revealed that the majority of cases in 2014/2015 were due to two major outbreak scenarios, i.e. two *M. hyopneumoniae* sequence types, each outbreak involving six individual production farms in five to six different Cantons (states), respectively. Moreover, by comparison of archived sequences some sequence types were observed over ten years demonstrating their persistence over a long time and the possible partial failure of eradication elimination measures in Switzerland. Insufficient sanitation on affected farms and subsequent animal transport of symptomless infected pigs could lead to recurrent outbreaks. Wild boar harbor identical strains found in EP outbreaks but solid data are missing to assign a role as reservoir to this wild animal. Implementing a monitoring scheme for *M. hyopneumoniae* in wild boar in combination with genotyping of all available samples from domestic pigs could direct responsible authorities to possible gaps and deficiencies of control measures taken for combating enzootic pneumonia. With the newly installed PubMLST database sequence types for *M. hyopneumoniae* are now available and allow tracing back strains on the international level.

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. Adhikary S, Nicklas W, Bisgaard M, Boot R, Kuhnert P, Waberschek T, Aalbaek B, Korczak B, Christensen H. 2017. *Rodentibacter* gen. nov. including *Rodentibacter pneumotropicus* comb. nov., *Rodentibacter heylii* sp. nov., *Rodentibacter myodis* sp. nov., *Rodentibacter ratti* sp. nov., *Rodentibacter heidelbergensis* sp. nov., *Rodentibacter trehalosifermentans* sp. nov., *Rodentibacter rarus* sp. nov., *Rodentibacter mrazii* and two genomospecies. Int J Syst Evol Microbiol **67**:1793-1806, *doi:* 10.1099/ijsem.0.001866.
2. Alinaitwe L, Kakooza S, Eneku W, Dreyfus A, Rodriguez-Campos S. 2017. Case of clinical canine leptospirosis in Uganda. Vet Rec Case Reports **5**:e000484, *doi:* 10.1136/vetreccr-2017-000484.
3. Andreis SN, Perreten V, Schwendener S. 2017. Novel beta-Lactamase *bla*$_{\text{ARL}}$ in *Staphylococcus arlettae*. mSphere **2**, *doi:* 10.1128/mSphere.00117-17.
4. Asimaki E, Nolte O, Overesch G, Strahm C. 2017. A dangerous hobby? *Erysipelothrix rhusiopathiae* bacteremia most probably acquired from freshwater aquarium fish handling. Infection **45**:557-562, *doi:* 10.1007/s15010-016-0966-z.
5. Baez M, Collaud A, Espinosa I, Perreten V. 2017. MRSA USA300, USA300-LV and ST5-IV in pigs, Cuba. Int J Antimicrob Agents **49**:259-261, *doi:* 10.1016/j.ijantimicag.2016.12.001.
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9. Brand P, Gobeli S, Perreten V. 2017. Pathotyping and antibiotic resistance of porcine enterovirulent *Escherichia coli* strains from Switzerland (2014-2015). Schweiz Arch Tierheilkd **159**:373-380, *doi:* 10.17236/sat00120.
10. Cotting K, Strauss C, Rodriguez-Campos S, Rostaher A, Fischer NM, Roosje PJ, Favrot C, Perreten V. 2017. *Macrococcus canis* and *M. caseolyticus* in dogs: occurrence, genetic diversity and antibiotic resistance. Vet Dermatol **28**:559-e133, *doi:* 10.1111/vde.12474.
11. Delaude A, Rodriguez-Campos S, Dreyfus A, Counotte MJ, Francey T, Schweighauser A, Lettry S, Schuller S. 2017. Canine leptospirosis in Switzerland-A prospective cross-sectional study examining seroprevalence, risk factors and urinary shedding of pathogenic leptospires. Prev Vet Med **141**:48-60, *doi:* 10.1016/j.prevetmed.2017.04.008.
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13. Dona V, Bernasconi OJ, Pires J, Collaud A, Overesch G, Ramette A, Perreten V, Endimiani A. 2017. Heterogeneous Genetic Location of *mcr-1* in Colistin-Resistant *Escherichia coli* Isolates from Humans and Retail Chicken Meat in Switzerland: Emergence of *mcr-1*-Carrying IncK2 Plasmids. Antimicrob Agents Chemother **61**, *doi:* 10.1128/AAC.01245-17.
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15. Fernandez-Aguilar X, Cabezon O, Frey J, Velarde R, Serrano E, Colom-Cadena A, Gelormini G, Marco I, Mentaberre G, Lavin S, Lopez-Olvera JR. 2017. Long-term dynamics of *Mycoplasma conjunctivae* at the wildlife-livestock interface in the Pyrenees. PLoS One **12**:e0186069, *doi:* 10.1371/journal.pone.0186069.
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18. Ferri F, Zini E, Auriemma E, Castagnaro M, Coppola LM, Peano A, Martella V, Decaro N, Kuhnert P, Ferro S. 2017. Splenitis in 33 Dogs. Vet Pathol **54**:147-154, *doi:* 10.1177/0300985816653989.
19. Gelormini G, Gauthier D, Vilei EM, Crampe JP, Frey J, Ryser-Degiorgis MP. 2017. Infectious keratoconjunctivitis in wild *Caprinae*: merging field observations and molecular analyses sheds light on factors shaping outbreak dynamics. BMC Vet Res **13**:67, *doi:* 10.1186/s12917-017-0972-0.
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22. Haas K, Overesch G, Kuhnert P. 2017. A Quantitative Real-Time PCR Approach for Assessing *Campylobacter jejuni* and *Campylobacter coli* Colonization in Broiler Herds. J Food Prot **80**:604-608, *doi:* 10.4315/0362-028X.JFP-16-395.
23. Hamza E, Kittl S, Kuhnert P. 2017. Temporal induction of pro-inflammatory and regulatory cytokines in human peripheral blood mononuclear cells by *Campylobacter jejuni* and *Campylobacter coli*. PLoS One **12**:e0171350, *doi:* 10.1371/journal.pone.0171350.
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49. Zeeh F, Nathues H, Frey J, Muellner P, Fellstrom C. 2017. A review of methods used for studying the molecular epidemiology of *Brachyspira hyodysenteriae*. Vet Microbiol **207**:181-194, *doi:* 10.1016/j.vetmic.2017.06.011.

5.2 Book Chapters

- N/A

5.3 Other Publications

- Delaude A, Rodriguez-Campos S, Dreyfus A, Francey T, Schweighauser A, Schuller S. The role of the dog in the epidemiology of leptospirosis in Switzerland - seroprevalence and urinary shedding of pathogenic leptospires. J Vet Intern Med 2017;31:186–270

- Overesch G. (2017) Nachweis von Methicillin-resistenten *Staphylococcus aureus* (MRSA) und *Staphylococcus pseudintermedius* (MRSP) bei Schweizer Tierärztinnen

und Tierärzten 2017. Studie in Zusammenarbeit mit der Schweizer Gesellschaft für Tierärzte (GST)

5.4 Press Releases and Broadcasting

Novel Antibiotic Resistance gene in Milk

The discovery of the novel methicillin resistance gene *mecD* published in Scientific reports (Schwendener S, Cotting K, Perreten V. 2017. Sci Rep. **7**:43797) has been released to the press through the Corporate Communication of the University of Bern, April 27th 2017. (www.unibe.ch/news/media_news/media_relations_e/media_releases/2017_e/media_releases_2017/novel_antibiotic_resistance_gene_in_milk/index_eng.html)

The communication was mentioned in more than 110 different newspapers and on-line journals and on television news:

RTS Radio Télévision Suisse Romande, Téléjournal (News) 19h30, November 11th 2017

RSI Radiotelevisione svizzera di lingua italiana, Telegiornale (News) 20h, November 11th, 2017

Trop d'antibiotiques tue les antibiotiques!

RTS Radio Télévision Suisse Romande – A Bon Entendeur, November 14th, 2017

Superkeime – Die tödlichen Feinde

NZZ format, 3SAT, May 25th, 2017

6 Graduations and Visting Scientists

6.1 PhD Degrees

N/A

6.2 Dr. vet. med. Degrees

Name of student: Sabrina Nathalie Andreis.

Title of Thesis: Identification of β-lactam and macrolide resistance genes in coagulase-negative staphylococci.

Supervisors: Vincent Perreten and Sybille Schwendener

Abstract: Coagulase-negative staphylococci (CNS) isolated from bovine mastitis milk samples in Switzerland were screened for the presence of novel antibiotic resistance genes by phenotypic and genotypic comparative analysis. Whole genome sequencing of the penicillin and macrolide-resistant *Staphylococcus arlettae* strain SAN1670 revealed a novel β-lactamase operon consisting of the β-lactamase bla_{ARL}, the antirepressor $blaR1_{\mathrm{ARL}}$ and the repressor $blaI_{\mathrm{ARL}}$. Functionality of bla_{ARL} was demonstrated by gene expression in *Staphylococcus aureus*. The bla_{ARL} operon was chromosomally located in SAN1670 and present in nine additional unrelated strains suggesting intrinsic penicillin resistance in *S. arlettae*. Furthermore, GenBank search revealed more unique potential β-lactamases in *Staphylococcus* species. Strain SAN1670 also carried an alternative *msr*(A) gene associated with macrolide resistance. It encodes a Msr(A)-like protein of 488 amino acids that contains two ATP-binding cassette (ABC) transporter signatures typical for class 2 ABC proteins. Comparative sequence analysis with other *S. arlettae* genomes indicates that the *msr*(A)-like gene was inserted into a region of the *S. arlettae* chromosome which shows diversity amongst the different *S. arlettae* strains. This study revealed novel antibiotic resistance genes in CNS from bovine milk, emphasising once again the role of animals as reservoir for antibiotic-resistant CNS. Targeted use of antibiotics is therefore recommended to reduce the risk of selecting new resistances that might be mobilised and spread to other bacteria.

Winner of the Faculty Price for best thesis 2017.

Name of student: Kerstin Cotting

Title of Thesis: Genetic characteristics and antibiotic resistance of opportunistic pathogenic gram-positive cocci from the skin of dogs.

Supervisor: Vincent Perreten

Abstract: Gram-positive bacteria are part of the normal microbiota of dogs and some of them can cause infections. The aim of this thesis was to study antibiotic resistance and clonality of *Macrococcus* isolated from non-infection and infection sites of dogs, and transmission of *S. pseudintermedius* within dog families. In the first study, samples were obtained from 162 healthy dogs and from 6 dogs with *Macrococcus* infections. Macrococci were identified by

MALDI TOF MS, 16S rRNA sequencing and *nuc*-PCR. Antibiotic resistance was determined by the measurement of the MIC, microarray, PCR and sequencing. A novel MLST scheme was developed to determine sequence type (ST). *M. canis* and *M. caseolyticus* were found in both skin and infections. The 22 *M. canis* strains belonged to 20 different STs and the 7 *M. caseolyticus* to 3 STs. Decreased susceptibility to antibiotics could mostly be associated with the detection of known genes with *mecB* being the most frequent. In the second study, *S. pseudintermedius* was isolated from skin and infection sites of 147 dogs including bitches, male dogs and puppies of 29 dog families. Clonality was determined by MLST and PFGE, resistance by MIC determination. Members of a family harbored either clonally related or unrelated *S. pseudintermedius* and in two cases, the clone acquired from the mother developed into an infection. This study gives a first insight into the occurrence and genetic characteristics of antibiotic-resistant *Macrococcus* from dogs and shows mother-to-puppies-to-infection transmission of specific *S. pseudintermedius* clones.

Name of student: Piera Alena Brand

Title of Thesis: Pathotyping and antibiotic resistance of porcine enterovirulent Escherichia coli strains from Switzerland (2014-2015).

Supervisor: Vincent Perreten

Abstract: Porcine *E. coli* (n=131) were isolated from the gut of pigs raised in Switzerland and suffering from diarrhea. Half of the *E. coli* isolates possessing virulence factors could not be assigned to any of the 19 serotypes tested, but contained toxins and adhesins similarly to the sero-typable *E. coli* isolates. The most prevalent *E. coli* serotypes found was K88ac (18%). The combination of toxins EAST-1, STb and LT-I and adhesin F4 characterizing ETEC was the most frequent. The shigatoxin Stx2e (STEC) and intimin Eae (EPEC) were also detected, but less frequently. Predominant resistances were those to tetracycline (50% of the isolates), sulfamethoxazole (49%), and trimethoprim (34%). All isolates were susceptible to 3rd generation cephalosporins, carbapenems, colistin and tigecycline. Resistant isolates harbored resistance mechanisms conferring resistance to antibiotics which are commonly used in pig husbandry, except for colistin. A careful identification of the causative agent and antibiotic susceptibility testing is highly recommended for targeted therapy and prudent use of antibiotics.

Name of student: Philipp Schnydrig

Title of Thesis: Analysis of neglected bacterial pathogens in abortion in small ruminants.

Supervisor: Sabrina Rodriguez Campos

Abstract: Abortion in small ruminants can be caused by a broad range of abortifacients including several zoonotic pathogens. We studied 41 ovine and 36 caprine abortions by bacteriological and mycological culture for culturable classical and opportunistic abortive agents, by real-time PCR for *C. burnetii*, *C. abortus*, pathogenic *Leptospira* spp. and in 30 cases

also for *Toxoplasma gondii* and *Neospora caninum*. In 40 cases serum of the dam was also analyzed to detect antibodies against *B. melitensis, C. burnetii*, *C. abortus* and *Leptospira* spp. In 37 cases a pathological examination was also carried out. In a further study we assessed the role of *Salmonella enterica* subsp. *diarizonae* 61:(k):1,5,(7), which is considered host-adapted to sheep, in abortion in small ruminants by PFGE. The clustering of the clinical strains with isolates from normal microbiota suggests a role of *S.* IIIb 61:(k):1,5,(7) as a classic opportunistic pathogen. Our study shows that the highest clarification rate can only be achieved by a combination of molecular and pathological analysis and evidences the role that multi-infections play as cause of abortion. The inclusion of the opportunistic pathogens in abortion diagnostics is key to gain a better comprehension about to the role of opportunistic pathogens in abortion.

6.3 Master Degrees

Name of student: Aurélien Nigg

Title of Thesis: Characterization of *mecD* islands in genetically diverse methicillin-resistant *Macrococcus caseolyticus* strains from cattle.

Supervisors: Sybille Schwendener, Vincent Perreten

Abstract: The novel methicillin resistance gene *mecD* has been recently discovered in *M. caseolyticus* in Switzerland. In this study, we established three multiplex PCRs for rapid characterization of *mecD* resistance islands (McRI$_{mecD}$) in methicillin-resistant *M. caseolyticus* strains. Twelve *mecD*-positive isolates from cattle were analyzed with this method and the structure of their *mecD* islands compared with those of the two already known islands, namely McRI$_{mecD}$-1 and McRI$_{mecD}$-2. Furthermore, the sequence type (ST) and resistance profile of the isolates were determined. The majority of the strains contains resistance islands that are similar to either McRI$_{mecD}$-1 (n=8) or McRI$_{mecD}$-2 (n=3). A novel McRI$_{mecD}$-3 was found in one strain (ST 7) that contains a complete *mecD* operon combined with the left part of McRI$_{mecD}$-2 and the right part of the McRI$_{mecD}$-1. McRI$_{mecD}$-1 was carried by strains presenting different STs (ST 5, ST 8, and ST 9) and beside β-lactam resistance (*mecD*), additional resistances to tetracycline (n=6, [*tet*(L), *tet*(M)]), aminoglycosides (n=7, [*aph(2')-Ia-aac(6')-Ie*, *str*]), trimethoprim (n=4, [*drf*(K), *dfr*(D)] and macrolide (n=2, [*erm*(B)]) were found. McRI$_{mecD}$-2 was only found in *M. caseolyticus* with ST 6 that were resistant to β-lactams (n=3, [*mecD*]), tetracycline (n=2, [*tet*(L)]) and aminoglycosides (n=2, [*str*]). Our results show that different *mecD*-positive *M. caseolyticus* clones are circulating in Switzerland. They were isolated from bovine mastitis milk as well as from the nose of healthy calves, which might indicate transmission though contaminated milk.

Name of student: Nicole Niederhäusern

Title of Thesis: Direct identification of urine by MALDI TOF Mass Spectrometry.

Supervisor: Stefanie Gobeli-Brawand

Abstract: Urinary tract infections (UTI) are a common reason for a consultation in a small animal practice. If UTI is expected, a complete urinalysis is an important part of the diagnosis. Usually the clinicians collect a urine sample and send it to a bacteriological laboratory to perform culture and antimicrobial resistance testing. At the laboratory the analysis usually takes 24 to 48 hours. During the long time of analysis a specific treatment is not possible. The clinicians have to wait for the results or start with an empirical therapy. This may cause delayed convalescence of the patient or increase the risk of development of antibiotic resistance, if treatment is initiated with an inconvenient antibiotic. Several papers in human medicine describe faster identification protocols by means of Matrix-assisted laser desorption – time-of-flight (MALDI-tof) mass spectrometry used amongst others for urine and blood samples. The aim of this master thesis was to test some of these protocols for veterinary samples, especially for urine samples of dogs and cats. The time of analysis would be much shorter, thus allowing a faster and still precise treatment. Overall 64 urine samples from dogs and cats were analysed. The samples were collected in different veterinary clinics and practices and sent to the Center for Zoonoses, Animal Bacterial Diseases and Antimicrobial Resistance (ZOBA) in Berne to clarify a clinical suspicion of UTI. The urine samples were analysed using a centrifugation protocol. For this purpose, the samples were centrifuged several times and the received pellets were analysed by MALDI-tof mass spectrometry. In addition about half of the urine samples were analysed with a short growth protocol. For this purpose, the urine samples were cultivated on blood-agars plates for 5 hours and then analysed using MALDI-tof mass spectrometry. The results of both methods were then compared to the results of the routine analysis. In both protocols, the number of colony forming units (CFU) was essential. Low bacterial counts hinder the correct identification. Overall, the diagnostic specificity was in both protocols very high with 100% for both the centrifugation and the short growth protocol, while the diagnostic sensitivity was rather low with 54.3 % for the centrifugation protocol and 71.4 % for the short growth protocol. Furthermore, a few milk samples (n=8) were analysed using the identical centrifugation protocol in combination with an extraction. Identification of the causing agent was not possible in any of the samples. There were no additional tests performed, as this master thesis was focussed on the analysis of urine samples. For analysis of urine samples both the centrifugation and the short growth protocol seemed promising. But further experiments are necessary to improve the diagnostic sensitivity. In particular, larger amounts of urine and therewith higher number of colony forming units (CFU) should be processed.

Name of student: Claudia Stauffer

Title of Thesis: Epizootic disease pathogens can be transmitted in various ways, for instance by faeces, droplets, bloodsucking arthropods, wild animals, contaminated material and so on.

Supervisor: Kathrin Summermatter

Diagnostics and Expertise: Stefanie Gobeli-Brawand

Abstract: Since the implementation of training and the biosafety documentation in handling animals were poor, the salmonella outbreak of the Vetsuisse ruminants clinic in 2016 offers a

good opportunity to analyze these retrospectively and to identify the shortcomings of the taken biosafety measures. The critical points that were observed deal with different procedures humans, animals, feed or the manure undergo as well as the given infrastructure, equipment and personnel. The aim of this work was to define biosafety measures emerging from these findings in order to better deal with or even prevent future outbreaks.

Name of student: Tobias Hidber

Title of Thesis: Monitoring of antimicrobial resistance in veterinary pathogens – significance of choosing the appropriate isolates

Supervisor: Gudrun Overesch

Abstract: Antimicrobial resistance represents a challenge in human and veterinary medicine. Important tools to measure the resistance situation are monitoring systems. The applied methods for monitoring program differ strongly between organisation and countries, which hampers the comparability of the results. Choosing the isolates has a major impact on the results. The perception is, that strains isolated from animals that previously underwent antimicrobial treatment, show a higher resistance rate than isolates of untreated animals. The goal of our study was, to compare the resistance rate for isolates from untreated, pretreated and animals with unknown treatment status in order to assess the suitability of these isolates for monitoring program. For this purpose, isolates from routine diagnostic samples submitted to the Center of Zoonoses, Animal Bacterial Diseases and Antimicrobial Resistance (ZOBA) were analyzed. *E.coli* was isolated from urine samples of diseased dogs and identified by Matrix-assisted laser desorption/ionization Time-of-flight mass spectrometry (MALDI TOF MS). The minimal inhibitory concentration (MIC) against several antimicrobials was tested using the broth microdilution method according to international standards. Resistance rates and multi drug resistance (MDR) rates were determined and tested for statistical significance. The resistance rate and the MDR rate of isolates tend to increase from untreated dogs to the group with unknown treatment status and the pretreated group, partially with statistical significant differences. Therefore, isolates from pretreated animals should not be used for monitoring purposes. If isolates from animals with unknown treatment status could contribute to monitoring program need further investigation.

6.4 Visiting Scientists

Name of student: Pattrarat Chanchaithong (Chulalongkorn University, Bangkok, Thailand)

Name of Research Project: Characterization of extensively drug-resistant community-acquired *Acinetobacter baumannii* and of methicillin-resistant *Macrococcus canis* from dogs

Supervisor: Vincent Perreten and Sybille Schwendener

7 Scientific Meetings Organized, Keynotes given and Grants Awarded

7.1 Scientific Meetings Organized by IVB Staff

N/A

7.2 Keynote Lectures Given by IVB Staff

- Jörg Jores, 13.-15. September 2017, Bad Staffelstein/Kloster Banz, Germany, 36th Meeting of the of Veterinary Diagnostics Working Group (AVID) of the German Veterinary Association, Title: "*Mycoplasma*-caused pleuropneumonia of ruminants in Africa"
- Jörg Jores, 21st September 2017, Bern-Liebefeld, 18. Informationsveranstaltung für veterinärmedizinische Diagnostiklaboratorien, Federal Food Safety and Veterinary Office Switzerland, Title: "Epizootic control in East Africa and the development of novel diagnostic tests and vaccines "
- Sabrina Rodriguez Campos, 21st September 2017, Bern-Liebefeld, 18. Informationsveranstaltung für veterinärmedizinische Diagnostiklaboratorien, Federal Food Safety and Veterinary Office Switzerland, Title: "Leptospirosis – activities of the reference laboratory (d)"
- Vincent Perreten, 22 – 24 March 2017. Discovery of novel antibiotic resistance genes from livestock. The 16th Chulalongkorn University Veterinary Conference 2017, Queen Sirikit National Convention Center, Bangkok, Thailand
- Gudrun Overesch, 22th June 2017, ETH Zurich, 50. Jahrestagung der Schweizer Gesellschaft für Lebensmittelhygiene (SGLH), Title: "Activities of the Center for bacterial zoonoses and epizootics and antimicrobial resistance (ZOBA) & health risks of Swiss meat? (d)"
- Gudrun Overesch, 17th November 2017, Bern-Liebefeld, Symposium Strategie Antibiotikaresistenzen(StAR) – was geht das Kleintierärzte an?, Federal Food Safety and Veterinary Office Switzerland, Title: "Diagnostics – how to interpretate culture results and antibiograms (d)"

7.3 Competitive Grants Awarded

- Donor: Vaccine challenge fund from IDRC (Canada), Project No: 108625-001; Project title: 'Development of a novel vaccine for contagious caprine pleuropneumonia based on a fast-growing *Mycoplasma feriruminatoris* chassis'; Duration: 11/2017-10/2019; Principal Investigator: Jörg Jores; Partners: J. Craig Venter Institute (USA), INRA (France)
- Donor: Swiss National Science Foundation (SNSF) Project No: 40AR40_174273 within the framework of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR); Project title: "Risk of companion animal to human transmission of antimicrobial resistance during different types of animal infection"; Duration 6/2017-5/2020. Contributor for Switzerland: Vincent Perreten. International contributers: Faculty of Veterinary Medicine, University of Lisbon, Portugal; Institute of Microbiology and Epizootics, Center for Infection Medicine, Department of Veterinary Medicine, Freie Universität Berlin; Ontario Veterinary College, University of Guelph, Ontario, Canada; Department of Clinical Science and

Services, The Royal Veterinary College, North Mymms, Hatfield, Hertfordshire, United Kingdom.

- Donor: Federal Food Safety and Veterinary Office (FSVO); Project title: "*Dichelobacter nodosus* in domestic ruminants, South American camelids and free-ranging wildlife in Switzerland - assessing prevalence in potential hosts to design targeted disease control measures", Duration: 01/2017-02/2019; First Co-applicant: Stefanie Gobeli Brawand, , Partners: Clinic for Ruminants and Centre for Fish and Wildlife Health, University of Bern (Switzerland).

7.4 Other funding

- Donor: Federal Food Safety and Veterinary Office (FSVO); Project title: "Determination of minimal inhibitory concentrations (MICs) of relevant pathogens for the establishment of epidemiological cut offs (ecoffs) according to EUCAST", Duration: 1/2017-12/2017; Principal Investigator: Gudrun Overesch, Institute of Veterinary Bacteriology, University of Bern (Switzerland)
- Donor: Federal Food Safety and Veterinary Office (FSVO); Project title: "MRSA in a One Health prospective", Duration: 12/2017-06/2018; Principal Investigator: Gudrun Overesch, Institute of Veterinary Bacteriology, University of Bern (Switzerland)

8 Organization Chart (Organigram)