



Jahresbericht - Annual Report 2014

Research, ZOBA

Institut für Veterinär-Bakteriologie, Vetsuisse Fakultät Universität Bern

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1. Preface

The present annual report gives a short overview on the highlight activities of the Institute. The year 2014 was a most successful year for all units of the Institute of Veterinary Bacteriology, University of Bern. The scientific output was particularly beneficial as revealed by the large number of publications and collaborative studies. In order to cope with the future exigent requirements in Veterinary Microbiology, the Institute started a residency program for the preparation to become Diplomate of the American College of Veterinary Microbiology (ACVM). In the field of basic and applied research the groups successfully acquired new research projects and continued their work on the current projects. The detection antibiotic resistance genes in different animal, food, and human environments as well as the identification of new antibiotic resistance genes and their mode of transmission made an important contribution to the understanding of basic biological mechanisms which are of high societal impact in the view of the current antibiotic-resistance problematic that has reached all countries around the globe. Scientists from the Institute acted as experts and guest speakers on many international and national congresses on bacterial pathogenesis and antibiotic resistance.

For the first time, the Institute made significant scientific and technical contributions in the field of ovine footrot which led finally to a rational approach to assess sheep as carriers of the virulent infectious agent *Dichelobacter nodosus*. This study would not have happened without the fruitful discussions with the research group of the Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics at Monash University, where I had the honour to be member of the scientific advisory board.

By integrating basic and applied research with development of novel diagnostic methods and conducting advanced training and continuous education programs, the staff of the Institute of Veterinary Bacteriology, contribute to animal health preservation, which is a main task of the Vetsuisse Faculty.

Bern, 21 June 2015

Joachim Frey

2. Research Units

2.1. Molecular Mechanisms of Bacterial Virulence

Joachim Frey and Peter Kuhnert

2.1.1. Molecular genetic analysis of *Dichelobacter nodosus* proteases in clinical samples from European sheep

Anna Stäuble, Adrian Steiner, Peter Kuhnert, Lea Normand, Joachim Frey

A collaborative study with the Clinic for Ruminants, Department of Clinical Veterinary Medicine, Vetsuisse-Faculty, Bern.

Ovine footrot is a highly infectious disease caused by the gram-negative bacterium *Dichelobacter nodosus* (formerly known as *Bacteroides nodosus*). It is present in many countries and has recently been reported in Europe. This debilitating disease is considered to be one of the most important causes of lameness in sheep flocks. Apart from the animal ethics issues evoked by the painful condition, the lameness is of considerable economic importance. In the alpine area, foraging involves major walking distances. As a consequence, the disease is responsible for losses in meat, wool and milk production, and it increases labour and management efforts relating to treatment and eradication.

Footrot is spread in Switzerland. In the Alps, where sheep from various flocks spend their summer on common pastures, cross contaminations by sheep from flocks with footrot is a major cause of spread of the disease in the country. However, efficient control or eradication of the disease in alpine areas is not possible as a rapid and efficient way of detecting infected animals, in particular animals at the early stage of infections where clinics are unapparent is inexistent. However, a reliable and rational diagnostic method for footrot is requested in order to become an officially controlled epidemic.

The current project on *Dichelobacter nodosus* aimed at getting solid fundamental scientific knowledge on virulence genes of *D. nodosus*. This finalized in a second stage in the development of a rational, robust method for early diagnosis of footrot, taking into account that pastures are not easily accessible and hence diagnostic samples need to be designed for transport without particular precautions such as cooling or fast shipping.

In infected animals *D. nodosus* colonises the damaged interdigital skin and is found in large quantities in the superficial layers of the early footrot lesion. Macroscopically, the condition is characterised by necrotising inflammation of the interdigital skin; a pasty foul smelling scum accumulates and necrotic separation of the horn wall from underlying tissue occurs. Clinical presentations vary and are classified with different scoring systems. Farming management and favourable environmental factors

influence the spread and progression of the disease. However, it is the nature of the causative bacterial strain which is decisive for the initiation and potential severity of an outbreak.

Extracellular subtilisin-like serine proteases (or subtilases) are commonly produced as pre-pro-precursors in a wide variety of organisms such as bacteria, archaea, fungi and eukaryotes. They are activated extracellularly by cleavage off the non-catalytic N-terminal pre-pro region and the C-terminal domain. Most of them have a broad substrate specificity and are required for either defence or growth on protein-containing substrates. This protein digesting process, as a source of amino acids and energy precursors, has also been postulated for *D. nodosus*. More importantly, however, the ability to produce subtilases is a key virulence factor in *D. nodosus*. Isolates are currently routinely distinguished by the elastase test and by the gelatine-gel test; they measure quantitative elastase activity and protease thermostability, respectively. Virulent strains produce the more heat stable acidic proteases AprV2 and AprV5 and the basic protease BprV; more benign strains produce the less thermostable enzymes AprB2, AprB5 and BprB. AprV2 is essential for virulence as confirmed recently by construction of isogenic protease mutants of a virulent reference strain.

In the present study we have analysed the alleles of the genes of the major extracellular virulent proteases AprV2, AprV5, BprV, and benign proteases AprB2, AprB5, and BprB of *D. nodosus* both from healthy and from footrot-affected sheep flocks in Switzerland, France, Germany and Norway.

Our study reveals the alleles of three major protease genes of *D. nodosus* present in Europe. Extracellular subtilases are essential enzymes in the pathogenesis of footrot. They are involved in the characteristic tissue destructive features of the disease. We used clinical material from sheep either suffering from or in contact with footrot, and clinical material and *D. nodosus* isolates from sheep of disease-free flocks originating from four European countries. By virulotyping a large number of strains, based on the gene sequences of *aprV2*, *aprV5*, *bprV*, *aprB2*, *aprB5* and *bprB* (Kennan et al. 2010 PLOS Pathogens 6:e1001210) we found that *aprV2* is the most reliable indicator for virulence. Molecular genetic analysis of the *aprV2* and *aprB2* gene sequences substantiate the prominent role of the allelic differences TA/CG at nt 661/662; the corresponding aa change Tyr92Arg distinguishes between the thermostable protease AprV2 in virulent and the thermos-labile AprB2 in benign *D. nodosus*. No particular genetic marker that would differentiate between virulent and benign *D. nodosus* strains could be evidenced in the other two elastase genes *aprV5* and *bprV*

Based on the molecular data a sensitive competitive real-time PCR method to detect and differentiate virulent from benign *D. nodosus* was developed and validated under field conditions. Particular attention was paid to the development of a buffer system that allows samples from ovine and caprine feet, that are taken with cotton swabs be conserved and transported without cooling system or other particular precautions.

The successful development of a reliable, solid and rational diagnostic for the infectious agent of footrot led finally to a successful parliamentary initiative to control and eradicate footrot in Switzerland

Publications:

Stäuble, A., Steiner, A., Normand, L., Kuhnert, P. and Frey, J. (2014) *Veterinary Microbiology* 168: 177-184.

Stäuble, A., Steiner, A., Frey J. and Kuhnert, P. (2014) *J. Clin. Microbiol.* 2014, 52:1228-1231.

2.1.2. Patho-genetics of *Clostridium chauvoei*

Joachim Frey in collaboration with Laurent Falquet Université de Fribourg and

Clostridium chauvoei is a highly pathogenic, histotoxic, anaerobic, endospore forming Gram-positive bacterium causing blackleg, a severe disease of cattle, sheep and other domestic animals. Blackleg is globally spread among ruminants specified primarily as a myonecrosis with high mortality causing significant losses in livestock production. *C. chauvoei* is one of the most pathogenic *Clostridium* species. Although *C. chauvoei* is mainly considered to be specific to ruminants, rare fatal cases of fulminant human gas gangrene and neutropenic enterocolitis caused by *C. chauvoei* have been reported and it is assumed that prevalence of *C. chauvoei* causing disease in humans may be higher than currently diagnosed. Infection of ruminants by *C. chauvoei* is caused by exposure of the animals to the pathogen present in form of spores in the soil of "poisoned" pastures. The infection by *C. chauvoei* leads to myonecrosis, oedemic lesions and fever, followed by lameness and death. Pathology of blackleg is mostly found in muscular tissue of infected animals from where the pathogen generally is isolated. Blackleg in cattle and sheep is controlled worldwide by commercial vaccines that consist of whole, inactivated bacteria and chemically toxoided culture supernatants. Furthermore, outer membrane proteins and flagella have been proposed as immunogens against *C. chauvoei* infections. The molecular mechanisms of pathogenicity of *C. chauvoei* in particular the spreading of this pathogen from the digestive tract where it is taken up to the muscle tissue where lesions are most abundant and where the pathogen is found at high amounts assumingly due to replication, is largely unknown. The genomic sequence of *Clostridium chauvoei*, the etiological agent of blackleg, a severe disease of ruminants, with high mortality specified by a myonecrosis reveals a chromosome of 2.8 million base-pairs and a cryptic plasmid of 5.5 kilo base-pairs. The chromosome contains the main pathways like glycolysis/gluconeogenesis, sugar metabolism, purine and pyrimidine metabolisms, but the notable absence of genes of the citric acid cycle and deficient or partially deficient amino acid metabolism for Histidine, Tyrosine, Phenylalanine, and Tryptophan. These essential amino acids might be acquired from host tissue damage caused by various toxins and by protein metabolism that includes 57 genes for peptidases, and several ABC transporters for amino acids import.

2.1.3. Complete genome sequences of virulent *Mycoplasma capricolum* subsp. *capripneumoniae*

A collaborative study with Dr. Jörg Jores, International of Livestock Research Institute (ILRI) Nairobi, Kenya and Dr. Laurent Falquet, Université de Fribourg.

In Kenya, goats account for more than 10,000,000 heads of livestock. Many resource-limited and livestock-dependent people especially in semi-arid areas keep goats for their livelihood. One of the major diseases affecting goat production systems because of its high mortality is contagious caprine pleuropneumonia (CCPP). CCPP is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*). *Mccp* belongs to the '*Mycoplasma mycoides* cluster' and is a particularly fastidious growing *Mycoplasma* species.. The disease features prominently in East-Africa, in particular Kenya, Tanzania and Ethiopia. CCPP endangers also wildlife thus affects not only basic nutritional resources of large populations but also expensively built up game resorts in affected countries. Here, we report the complete sequences of two *Mccp* strains: the type strain F38 and strain ILRI181 isolated from a recent outbreak in Kenya. Both genomes have a G+C content of 24% and a F38 a size of 1,016,760 bp and ILRI181 a size of 1,017,183 bp. The new data allow comparative genetics and represent a basic requirement for development of an efficient and safe vaccine against CCPP.

Publication: Falquet L, Liljander A, Schieck E, Gluecks I, Frey J, Jores J. (2014) Genome Announc. Oct 16;2(5). pii: e01041-14.

2.1.4. Characterisation of a new group of *Francisella tularensis* subsp. *holarctica* in Switzerland with altered antimicrobial susceptibilities.

Paola Pilo, Francesco Origi, Joachim Frey, in collaboration with the Centre for Fish and Wildlife

Francisella tularensis is a Gram-negative bacterium causing the zoonotic disease tularemia. The two clinically relevant subspecies are *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica*. Of the two, only the latter subspecies is present in Europe. Human infections mainly occur through inhalation, ingestion, or by direct contact with infected animal species and contaminated animal tissues, water and aerosols. Molecular analysis of *Francisella tularensis* subsp. *holarctica* isolates from humans and animals revealed the presence of two subgroups belonging to the phylogenetic group B.13 in Switzerland. This finding suggests a broader spread of this group in Europe than previously reported. Until recently, only strains belonging to the Western European cluster (group B.FTNF002-00) had been isolated from tularemia cases in Switzerland. The endemic strains belonging to group B.FTNF002-00 are sensitive to erythromycin, in contrast to the strains of the newly detected group B.13 that are resistant to this antibiotic. All the strains tested were susceptible to ciprofloxacin, streptomycin, gentamicin, nalidixic acid and chloramphenicol but showed reduced susceptibility to tetracycline when tested in a growth medium supplemented with divalent cations. The data show a previously undetected spread of group B.13

westwards in Europe, associated with changes in the antibiotic resistance profile relevant to treatment of tulareamia.

Publication: Origgi, F.C., Frey, J. and Pilo, P. (2014) *Eurosurveillance*, Volume 19: 29.
2.1.5. Source attribution of human *Campylobacter* infections

Peter Kuhnert, Sonja Kittl, Chantal Amar, Romie Jonas, in collaboration with Valentina Biancchini and Mario Luini, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Lodi, 26900, Italy

Campylobacter jejuni is the most important cause of bacterial gastroenteritis in humans. It is a commensal in many wild and domestic animals, including dogs. Whereas genotypes of human and chicken *C. jejuni* isolates have been described in some detail, only little information on canine *C. jejuni* genotypes is available. To gain more information on genotypes of canine *C. jejuni* and their zoonotic potential, isolates from routine diagnostics of diarrheic dogs as well as isolates of a prevalence study in non-diarrheic dogs were analyzed. Prevalence of thermophilic *Campylobacter* among non-diarrheic dogs was 6.3% for *C. jejuni*, 5.9% for *C. upsaliensis* and 0.7% for *C. coli*. The *C. jejuni* isolates were genotyped by multi locus sequence typing (MLST) and *flaB* typing. Resistance to macrolides and quinolones was genetically determined in parallel. Within the 134 genotyped *C. jejuni* isolates 57 different sequence types (ST) were found. Five STs were previously unrecognized. The most common STs were ST-48 (11.2%), ST-45 (10.5%) and ST-21 (6.0%). Whereas no macrolide resistance was found, 28 isolates (20.9%) were resistant to quinolones. ST-45 was significantly more prevalent in diarrheic than in non-diarrheic dogs. Within the common time frame of isolation 94% of the canine isolates had a ST that was also found in human clinical isolates. In conclusion, prevalence of *C. jejuni* in Swiss dogs is low but there is a large genetic overlap between dog and human isolates. Given the close contact between human and dogs, the latter should not be ignored as a potential source of human campylobacteriosis.

Campylobacter jejuni has gained more importance in Italy following the increased consumption of raw milk. The aim of a collaborative study with the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Lodi was therefore to further characterize *C. jejuni* strains isolated in Northern Italy from bulk tank milk, cattle and pigeons. In particular, *flaB* typing and sequence-based determination of quinolone and macrolide resistances were used. *flaB*-typing revealed 22 different types with one of them being novel and was useful to further differentiate strains with an identical sequence type and to identify a pigeon-specific clone. Macrolide resistance was not found, while quinolone resistance was detected in 23.3% of isolates. A relationship between specific genotypes and antibiotic resistance was observed, but was only significant for the clonal complex 206. Our data confirm that pigeons do not play a role in the spread of *C. jejuni* among cattle and they are not responsible for milk contamination. A relevant number of bulk milk samples was contaminated by *C. jejuni* resistant to quinolones, representing a possible source of human resistant strains.

Publications:

Amar, C., Kittl, S., Spreng, D., Thomann, A., Korczak, B.M., Burnens, A.P., Kuhnert P. (2014) Vet Microbiol. 168(1):124-30
Bianchini V, Luini M, Borella L, Parisi A, Jonas R, Kittl S, Kuhnert P. (2014) Int J Environ Res Public Health.11(7):7154-62

2.1.6. Outbreak investigations of enzootic pneumonia (*Mycoplasma hyopneumoniae*)

Peter Kuhnert and Gudrun Overesch

Mycoplasma hyopneumoniae is the major cause of enzootic pneumonia (EP) in domestic pigs, a disease with low mortality but high morbidity, having a great economic impact for producers. In Switzerland EP has been successfully eradicated, however, sporadic outbreaks are observed with no obvious source. Besides the possibility of recurrent outbreaks due to persisting *M. hyopneumoniae* strains within the pig population, there is suspicion that wild boars might introduce *M. hyopneumoniae* into swine herds. To elucidate possible links between domestic pig and wild boar, epidemiological investigations of recent EP outbreaks were initiated and lung samples of pig and wild boar were tested for the presence of specific genotypes by multilocus sequence typing (MLST). Despite generally different genotypes in wild boar, outbreak strains could be found in geographically linked wild boar lungs after, but so far not before the outbreak. Recurrent outbreaks in a farm were due to the same strain, indicating unsuccessful sanitation rather than reintroduction by wild boar. In another case outbreaks in six different farms were caused by the same strain never found in wild boar, confirming spread between farms due to hypothesized illegal animal transport. Results indicate presence of identical lineages of wild boar and domestic pig strains, and possible transmission of *M. hyopneumoniae* between wild boar and pig. However, the role of wild boar might be rather one as a recipient than a transmitter. More important than contact to wild boar for sporadic outbreaks in Switzerland is apparently persistence of *M. hyopneumoniae* within a farm as well as transmission between farms.

Publication. Kuhnert, P. and Overesch, G. (2014) Vet Microbiol. 174(1-2):261

2.1.7. *Frederiksenia canicola* gen. nov., sp. nov. isolated from dogs and human dog-bite wounds

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

Members of the *Pasteurellaceae* are frequently found in the oral cavity and upper respiratory tract of companion animals such as dogs and cats. They are mainly considered commensals, however, under certain circumstances they may also act as opportunistic pathogens. Species obtained from dogs and cats include *Pasteurella multocida*, *Pasteurella dagmatis*, *Pasteurella stomatis* and *Pasteurella oralis*. Two additional species are more restricted in their host-specificity, *Pasteurella canis* with

dogs and [*Haemophilus*] *felis* with cats. In humans the aforementioned species may cause wound infections inflicted by dog- or cat bites/scratches. Correct classification of these taxa has major impact on an unambiguous identification and is essential for proper medical treatment of patients, the development of preventive measures and the performance of epidemiological studies. Identification of these species, however, can be problematic as additional *Pasteurella*-like organisms have been reported from the same niche, like e.g. strains formerly known as Bisgaard taxon 16. Polyphasic analysis was therefore done on 24 strains of Bisgaard taxon 16 from five European countries and mainly isolated from dogs and human dog-bite wounds in order to properly classify them. The isolates represented a phenotypically and genetically homogenous group within the family *Pasteurellaceae*. Their phenotypic profile was similar to members of the genus *Pasteurella*. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) clearly identified taxon 16 and separated it from all other genera of *Pasteurellaceae* showing a characteristic peak combination. Taxon 16 can be further separated and identified by a RecN protein signature sequence detectable by a specific PCR. In all phylogenetic analyses based on 16S rRNA, *rpoB*, *infB* and *recN* genes, taxon 16 formed a monophyletic branch with intraspecies sequence similarity of at least 99.1%, 90.8%, 96.8% and 97.2%, respectively. Taxon 16 showed closest genetic relationship with *Bibersteinia trehalosi* as to the 16S rRNA gene (95.9%), the *rpoB* (89.8%) and the *recN* (74.4%), and with *Actinobacillus lignieresii* for *infB* (84.9%). Predicted genome similarity values based on the *recN* gene sequences between taxon 16 isolates and the type strains of known genera of *Pasteurellaceae* were below the genus level. Major whole cell fatty acids for the strain HPA 21^T are C_{14:0}, C_{16:0}, C_{18:0} and C_{16:1} ω7c/C_{15:0} iso 2OH. Major respiratory quinones are menaquinone-8, ubiquinone-8 and demethylmenaquinone-8. We propose to classify these organisms as a novel genus and species within the family of *Pasteurellaceae* named *Frederiksenia canicola* gen. nov., sp. nov. The type strain is HPA 21^T (=CCUG 62410^T =DSM 25797^T).

Publication: Korczak, B.M., Bisgaard, M., Christensen, H., Kuhnert, P. (2014) *Antonie Van Leeuwenhoek* 105(4):731

2.2. Molecular and Bacterial Epidemiology and Infectiology

Vincent Perreten

2.2.1. First report of OXA-23-mediated carbapenem resistance in sequence type 2 multidrug-resistant *Acinetobacter baumannii* associated with urinary tract infection in a cat.

In collaboration with Dr. Contança Pomba, Laboratory of Antimicrobial and Biocide Resistance, CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa (FMV-UL), Lisbon, Portugal, and Dr. Andrea Endimiani, Institute for Infectious Diseases, University of Bern, Bern, Switzerland.

Carbapenem resistance in multidrug-resistant *Acinetobacter baumannii* has been challenging human medicine and has also emerged in *Acinetobacter* spp. from animals; it is associated with the expression of OXA-23 in cattle and horses and NDM-1 in a porcine isolate. In this study, we describe a multidrug-resistant *A. baumannii* isolate producing OXA-23 in a urinary tract infection (UTI) in a cat. Strain FMV6475/09 belonged to sequence type 2, which has been associated with European clone II. In the same time frame, this worldwide-disseminated clone also containing *bla*_{OXA-23} on Tn2006 was endemic in Portuguese hospitals suggesting a possible human-to-animal transmission.

Publication: Pomba C, Endimiani A, Rossano A, Saial D, Couto N, Perreten V. (2014) Antimicrob. Agents Chemother. 58(2):1267-8.

2.2.2. Small colony variant of methicillin-resistant *Staphylococcus pseudintermedius* ST71 presenting as a sticky phenotype

In collaboration with Dr. Vincezo Savini, Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara, Italy, and Dr. Edoardo Carretto, Clinical Microbiology Laboratory, IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.

We first observed the phenomenon of small colony variants (SCVs) in a *Staphylococcus pseudintermedius* sequence type 71 (ST71) strain, isolated from a non-pet owner. Although we found that small-sized colonies share main features with *Staphylococcus aureus* SCVs, they nevertheless show a novel, particular, and sticky phenotype, whose expression was extremely stable, even after sub-cultivation.

Publication: Savini V, Carretto E, Polilli E, Marrollo R, Santarone S, Fazii P, D'Antonio D, Rossano A, Perreten V. (2014). J. Clin. Microbiol. 52(4):1225-7.

2.2.3. Occurrence and genetic characteristics of third-generation cephalosporin-resistant *Escherichia coli* in Swiss retail meat

Prevalence and genetic relatedness were determined for third-generation cephalosporin-resistant *Escherichia coli* (3GC-R-*Ec*) detected in Swiss beef, veal, pork, and poultry retail meat. Samples from meat-packing plants (MPPs) processing 70% of the slaughtered animals in Switzerland were purchased at different intervals between April and June 2013 and analyzed. Sixty-nine 3GC-R-*Ec* isolates were obtained and characterized by microarray, PCR/DNA sequencing, Multi Locus Sequence Typing (MLST), and plasmid replicon typing. Plasmids of selected strains were transformed by electroporation into *E. coli* TOP10 cells and analyzed by plasmid MLST. The prevalence of 3GC-R-*Ec* was 73.3% in chicken and 2% in beef meat. No 3GC-R-*Ec* were found in pork and veal. Overall, the *bla*_{CTX-M-1} (79.4%), *bla*_{CMY-2} (17.6%), *bla*_{CMY-4} (1.5%), and *bla*_{SHV-12} (1.5%) β-lactamase genes were detected, as well as other genes conferring resistance to chloramphenicol (*cmiA1-like*), sulfonamides (*suI*), tetracycline (*tet*), and trimethoprim (*dfrA*). The 3GC-R-*Ec* from chicken meat often harbored virulence genes associated with avian pathogens. Plasmid incompatibility (Inc) groups IncI1, IncFIB, IncFII, and IncB/O were the most frequent. A high rate of clonality (e.g., ST1304, ST38, and ST93) among isolates from the same MPPs suggests that strains persist at the plant and spread to meat at the carcass-processing stage. Additionally, the presence of the *bla*_{CTX-M-1} gene on an IncI1 plasmid sequence type 3 (IncI1/pST3) in genetically diverse strains indicates interstrain spread of an epidemic plasmid. The *bla*_{CMY-2} and *bla*_{CMY-4} genes were located on IncB/O plasmids. This study represents the first comprehensive assessment of 3GC-R-*Ec* in meat in Switzerland. It demonstrates the need for monitoring contaminants and for the adaptation of the Hazard Analysis and Critical Control Point concept to avoid the spread of multidrug-resistant bacteria through the food chain.

Publication: Vogt D, Overesch G, Endimiani A, Collaud A, Thomann A, Perreten V. (2014) *Microb. Drug Resist.* 20(5):485-94.

2.2.4. Nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among Swiss veterinary health care providers: detection of livestock- and healthcare-associated clones

In collaboration with Laboratory Laupeneck, Bern, Switzerland

We screened a total of 340 veterinarians (including general practitioners, small animal practitioners, large animal practitioners, veterinarians working in different veterinary services or industry), and 29 veterinary assistants for nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) at the 2012 Swiss veterinary annual meeting. MRSA isolates (n=14) were detected in 3.8% (95% CI 2.1-6.3%) of the participants whereas MRSP was not detected. Large animal practitioners were carriers of livestock-associated MRSA (LA-MRSA) ST398-t011-V (n=2), ST398-t011-IV (n=4), and ST398-t034-V (n=1). On the other hand, participants working with small animals harbored human healthcare-

associated MRSA (HCA-MRSA) which belonged to epidemic lineages ST225-t003-II (n=2), ST225-t014-II (n=1), ST5-t002-II (n=2), ST5-t283-IV (n=1), and ST88-t186-IV (n=1). HCA-MRSA harbored virulence factors such as enterotoxins, β -hemolysin converting phage and leukocidins. None of the MRSA isolates carried Panton-Valentine leukocidin (PVL). In addition to the methicillin resistance gene *mecA*, LA-MRSA ST398 isolates generally contained additional antibiotic resistance genes conferring resistance to tetracycline [*tet*(M) and *tet*(K)], trimethoprim [*dfrK*, *dfrG*], and the aminoglycosides gentamicin and kanamycin [*aac*(6')-Ie – *aph*(2')-Ia]. On the other hand, HCA-MRSA ST5 and ST225 mainly contained genes conferring resistance to the macrolide, lincosamide and streptogramin B antibiotics [*erm*(A)], to spectinomycin [*ant*(9)-Ia], amikacin and tobramycin [*ant*(4')-Ia], and to fluoroquinolones [amino acid substitutions in GrlA (S84L) and GyrA (S80F and S81P)]. MRSA carriage may represent an occupational risk and veterinarians should be aware of possible MRSA colonization and potential for developing infection or for transmitting these strains. Professional exposure to animals should be reported upon hospitalization and before medical intervention to allow for preventive measures. Infection prevention measures are also indicated in veterinary medicine to avoid MRSA transmission between humans and animals, and to limit the spread of MRSA both in the community, and to animal and human hospitals.

Publication: Wettstein Rosenkranz K, Rothenanger E, Brodard I, Collaud A, Overesch G, Bigler B, Marschall J, Perreten V. (2014). Schweiz. Arch. Tierheilkd. 156(7):317-25.

2.2.5. The novel macrolide-lincosamide-streptogramin B resistance gene *erm*(44) is associated with a prophage in *Staphylococcus xylosus*

A novel erythromycin ribosome methylase gene, *erm*(44), that confers resistance to macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics was identified by whole-genome sequencing of the chromosome of *Staphylococcus xylosus* isolated from bovine mastitis milk. The *erm*(44) gene is preceded by a regulatory sequence that encodes two leader peptides responsible for the inducible expression of the methylase gene, as demonstrated by cloning in *Staphylococcus aureus*. The *erm*(44) gene is located on a 53-kb putative prophage designated Φ JW4341-pro. The 56 predicted open reading frames of Φ JW4341-pro are structurally organized into the five functional modules found in members of the family *Siphoviridae*. Φ JW4341-pro is site-specifically integrated into the *S. xylosus* chromosome, where it is flanked by two perfect 19-bp direct repeats, and exhibits the ability to circularize. The presence of *erm*(44) in three additional *S. xylosus* strains suggests that this putative prophage has the potential to disseminate MLS_B resistance.

Publication: Wipf JR, Schwendener S, Perreten V. (2014) Antimicrob. Agents Chemother. 58(10):6133-8

2.2.6. Antibiotic resistance and phylogenetic characterization of *Acinetobacter baumannii* strains isolated from commercial raw meat in Switzerland

In collaboration with Dr. Andrea Endimiani, Institute for Infectious Diseases, University of Bern, Bern, Switzerland.

The spread of antibiotic-resistant bacteria through food has become a major public health concern because some important human pathogens may be transferred via the food chain. *Acinetobacter baumannii* is one of the most life-threatening gram-negative pathogens; multidrug-resistant (MDR) clones of *A. baumannii* are spreading worldwide, causing outbreaks in hospitals. However, the role of raw meat as a reservoir of *A. baumannii* remains unexplored. In this study, we describe for the first time the antibiotic susceptibility and fingerprint (repetitive extragenic palindromic PCR [rep-PCR] profile and sequence types [STs]) of *A. baumannii* strains found in raw meat retailed in Switzerland. Our results indicate that *A. baumannii* was present in 62 (25.0%) of 248 (CI 95%: 19.7 to 30.9%) meat samples analyzed between November 2012 and May 2013, with those derived from poultry being the most contaminated (48.0% [CI 95%: 37.8 to 58.3%]). Thirty-nine strains were further tested for antibiotic susceptibility and clonality. Strains were frequently not susceptible (intermediate and/or resistant) to third- and fourth-generation cephalosporins for human use (i.e., ceftriaxone [65%], cefotaxime [32%], ceftazidime [5%], and cefepime [2.5%]). Resistance to piperacillin-tazobactam, ciprofloxacin, colistin, and tetracycline was sporadically observed (2.5, 2.5, 5, and 5%, respectively), whereas resistance to carbapenems was not found. The strains were genetically very diverse from each other and belonged to 29 different STs, forming 12 singletons and 6 clonal complexes (CCs), of which 3 were new (CC277, CC360, and CC347). RepPCR analysis further distinguished some strains of the same ST. Moreover, some *A. baumannii* strains from meat belonged to the clonal complexes CC32 and CC79, similar to the MDR isolates responsible for human infections. In conclusion, our findings suggest that raw meat represents a reservoir of MDR *A. baumannii* and may serve as a vector for the spread of these pathogens into both community and hospital settings

Publication. Lupo A, Vogt D, Seiffert SN, Endimiani A, Perreten V. (2014) J. Food Prot. 77(11):1976-81.

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

Gudrun Overesch

In the division diagnostic and surveillance a total of 12530 samples, resp. animals were analysed. 100 analyses were conducted for the veterinary border control. Regarding the antimicrobial resistance monitoring program 2294 isolations for various pathogens, multi-resistant bacteria and indicator microorganisms were performed. Moreover 1118 analyses using the Minimal Inhibitory Concentration technique (MIC) were conducted. Furthermore, on behalf of the reference function for a broad variety of epizootics, our laboratory carried out 487 confirmations. Further details are shown in the tables below.

Diagnostic activities

Division	Number of samples/animals
Clinical material and mycology	1974 animals
Necropsy material and faeces	1070 animals
Surveillance	1540 animals
Bovine mastitis	2615 samples
Serology	2659 animals
Identification and molecular diagnostics	1936 samples
Antibiograms for diagnostics	736 analyses
Antimicrobial resistance monitoring (detection)	2294 analyses
Antimicrobial resistance monitoring (MIC*)	1118 analyses
Veterinary border control	100 analyses
Reference function	487 samples

* Minimal inhibitory concentration

3.1. Diagnostic Activity for Epizootics (Notifiable Animal Diseases)

Methods:

Micr	Microscopic examination
IF	Immunofluorescence
Cult	Culture
ELISA	Antibody detection by Enzyme-Linked Immunosorbent Assay
RBT	Antibody detection by Rose Bengal test
CFT	Antibody detection by complement fixation test
MAT	Antibody detection by the microscopic agglutination test
LF	Antibody detection by lateral flow test
PCR	Polymerase chain reaction
SEQ	Sequencing

3.1.1. Highly infectious diseases

Epizootic	Agent	Method	Animal	Total	negative	suspi- cious	positive
Contagious bovine pleuropneumonia	<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i>	Culture	cattle	0	0	0	0
		ELISA		0	0	0	0
		Immuno- blot		0	0	0	0

3.1.2. Diseases to be eradicated

Epizootic	Agent	Method	Animal	Total	nega- tive	suspi- cious	posi- tive
Anthrax	<i>Bacillus anthracis</i>	Micr	cattle	11	10	1	0
		Cult		11	11	0	0
	<i>Bacillus anthracis</i>	Micr	bison	1	1	0	0
		Cult		1	1	0	0
	<i>Bacillus anthracis</i>	Micr	swine	2	2	0	0
		Cult		2	2	0	0
Brucellosis	<i>Brucella abortus</i>	Micr	cattle	330	321	9	0
		RBT		8	7	0	1
		ELISA		707	706	0	1
		CFT		1	1	0	0
	<i>Brucella melitensis</i>	Micr	sheep/goat	24	20	4	0
		ELISA		106	103	0	3
		CFT		0	0	0	0
		RBT		2	2	0	0
	<i>Brucella abortus</i> / <i>Brucella melitensis</i>	Micr	diverse	14	13	1	0
				11	11	0	0
				11	11	0	0
			alpaca	10	9	0	1
			llama	1	1	0	0
			ibex/ chamois	8	8	0	0
		<i>Brucella suis</i>	Micr	swine	27	27	0
	RBT			854	845	0	9
	CFT			10	10	0	0
	<i>Brucella ovis</i>	ELISA	sheep	79	76	3	0
			others	0	0	0	0
	<i>Brucella canis</i>	Micr	dog	3	3	0	0
		LF		1	1	0	0

Epizootic	Agent	Method	Animal	Total	negative	suspicious	positive
Bovine Campylobacteriosis	<i>Campylobacter fetus</i> subspecies <i>venerealis</i>	Cult	cattle	891	737	154	0
		PCR		154	154	0	0
Infectious agalactia	<i>Mycoplasma agalactiae</i>	ELISA	goat	0	0	0	0

3.1.3. Diseases to be controlled

Epizootic	Agent	Method	Animal	Total	negative	suspicious	positive
Leptospirosis	<i>L. Australis</i>	MAT	cattle	481	478	3	0
			dog	286	158	19	91
			horse	25	13	7	5
			swine	80	65	8	7
	<i>L. Autumnalis</i>	MAT	cattle	5	5	0	0
			dog	266	183	49	34
			horse	25	17	7	1
			swine	3	3	0	0
	<i>L. Ballum</i>	MAT	cattle	14	14	0	0
			dog	7	7	0	0
			horse	0	0	0	0
			swine	13	13	0	0
	<i>L. Bataviae</i>	MAT	cattle	4	4	0	0
			dog	195	193	2	0
			horse	16	16	0	0
			swine	3	3	0	0
	<i>L. Bratislava</i>	MAT	cattle	5	5	0	0
			dog	268	169	19	80
			horse	25	15	6	4
			swine	86	76	4	6
	<i>L. Canicola</i>	MAT	cattle	481	481	0	0
			dog	207	145	36	26
			horse	19	15	3	1
			swine	25	25	0	0
<i>L. Grippotyphosa</i>	MAT	cattle	481	481	0	0	
		dog	268	219	38	11	
		horse	25	23	1	1	
		swine	36	36	0	0	

Epizootic	Agent	Method	Animal	Total	negative	suspi- cious	positive
Leptospirosis	<i>L. Hardjo</i>	MAT	cattle	484	462	6	16
			dog	202	201	1	0
			horse	16	16	0	0
			swine	34	34	0	0
	<i>L. Icterohaemo- rrhagiae</i>	MAT	cattle	481	481	0	0
			dog	266	244	20	2
			horse	25	25	0	0
			swine	25	25	0	0
	<i>L. Pomona</i>	MAT	cattle	481	480	1	0
			dog	265	202	36	27
			horse	25	22	1	2
			swine	36	36	0	0
	<i>L. Pyrogenes</i>	MAT	cattle	4	4	0	0
			dog	195	170	19	6
			horse	19	16	1	2
			swine	3	3	0	0
	<i>L. Sejroe</i>	MAT	cattle	482	466	14	2
			dog	198	198	0	0
			horse	16	16	0	0
			swine	3	3	0	0
	<i>L. Tarasosovi</i>	MAT	cattle	480	480	0	0
			dog	195	193	2	0
			horse	19	19	0	0
			swine	25	25	0	0
	<i>Leptospira</i> spp	PCR urine	cattle	8	1	7	0
			dog	3	2	0	1
			horse	1	0	0	1
			swine	2	2	0	0

Epizootic	Agent	Animal	Total	negative	susupi- cious	posi- tive	
Salmonellosis (Cult)		agouti	1	1	0	0	
		alpaca	1	1	0	0	
		ape	40	39	0	1	
	S. Enteritidis						1
		beaver	1	1	0	0	
		bird	23	23	0	0	
		cat	21	20	0	1	
	S. Veneziana						1

Epizootic	Agent	Animal	Total	negative	susupious	positive	
Salmonellosis		cattle	171	169	0	2	
	S. Enteritidis					1	
	S. Typhimurium					1	
		chameleon	1	1	0	0	
		chicken	8	7	0	1	
	S. Typhimurium					1	
		deer	6	6	0	0	
		dog	38	38	0	0	
		donkey	3	3	0	0	
		duck	6	6	0	0	
		gecko	1	0	0	1	
	S. Mowanjum					1	
		goat	14	14	0	0	
		hedgehoge	2	1	0	1	
	S. Enteritidis					1	
		horse	83	71	0	12	
	S. Enteritidis					7	
	S. Typhimurium					5	
		llama	1	1	0	0	
		mice	2	2	0	0	
		okapi	1	1	0	0	
		parrot	2	1	0	1	
	S. Typhimurium					1	
		rabbit	3	3	0	0	
		saurian	6	3	0	3	
	S. Mowanjum					1	
	S. Apapa*					1	
	S. Kisarawe					1	
		sheep	15	15	0	0	
		snake	21	11	0	10	
		<i>S. enterica</i> subsp. <i>arizonae</i> 44:z4, z24:-					1
		<i>S. enterica</i> subsp. <i>diarizonae</i> 35:-:z35*					1
		<i>S. enterica</i> subsp. <i>diarizonae</i> 61:r:z35*					1
	<i>S. enterica</i> subsp. <i>diarizonae</i> 61:r :z35*					1	
	<i>S. enterica</i> subsp. <i>diarizonae</i> rauh -:-:*					1	
	<i>S. enterica</i> subsp. <i>diarizonae</i> rauh -:i :-*					1	
	<i>S. enterica</i> subsp. <i>diarizonae</i> rauh -:z10:-*					1	
	<i>S. enterica</i> subsp. <i>houtenae</i> 11:z4,z32:-					1	
	S. Pomona					1	
	S. Wedding					1	

Epizootic	Agent	Animal	Total	negative	suspi- cious	posi- tive
Salmonellosis		swine	96	96	0	0
		tortoise	6	6	0	0
		wild animal	2	2	0	0
		zoo animal	22	22	0	0

* serotyping in human reference laboratory

Epizootic	Agent	Method	Animal	Total	negative	suspi- cious	positive
Contagious equine Metritis	<i>Taylorella equigenitalis</i>	Cult	horse	104	104	0	0
Enzootic pneumonia in swine	<i>Mycoplasma hyopneumoniae</i>	PCR Lung	swine	131	121	0	10
		PCR Nasal swab		46	34	6	6
		ELISA		35	34	1	0
Swine actinobacillosis	<i>Actinobacillus pleuropneumoniae</i>	Cult	swine		177	0	27
		PCR	BVII I BD+II CA		Serotyp 2	8	
			BVI I BD+II CA		Serotyp 7,12	8	
			BVI I BD+II CA+III CA+BD		Serotyp 2	9	
			BVI I BD+II CA+III CA+BD		Serotyp 4.6.8	2	
			ELISA ApxIV		38	6	0

3.1.4. Diseases to be monitored

Epizootic	Agent	Method	Animal	Total	nega- tive	suspi- cious	positive
Paratuberculosis	<i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i>	Micr	cattle	2	2	0	0
			diverse	5	5	0	0
Campylo- bacteriosis (thermotolerant)		Cult	dog	31	31	0	0
			cat	17	17	0	0
			monkey	28	28	0	0
			diverse	2	2	0	0
Listeriosis	<i>Listeria monocytogenes</i>	Cult	cattle	9	7	0	2
		Cult	sheep	0	0	0	0
Yersiniosis	<i>Yersinia</i> spp.	Cult	cattle	0	0	0	0
		Cult	diverse	39	37		2

Epizootic	Agent	Method	Animal	Total	negative	suspicious	positive
Caseous lymphadenitis in sheep and goats	<i>Corynebacterium pseudotuberculosis (ovis)</i>	Cult	goat	2	2	0	0
		Cult	sheep	2	0	0	2
Enzootic abortion in ewes (chlamydiosis)	<i>Chlamydophila abortus</i>	Micr	sheep	7	7	0	0
		ELISA		12	7	0	5
		PCR		4	4	0	0
		Micr	goat	6	6	0	0
		ELISA		7	5	0	2
		PCR		4	4	0	0
		Micr	cattle	138	134	4	0
		ELISA		5	2	0	3
	PCR		82	82	0	0	
Psittacosis	<i>Chlamydophila psittaci</i>	PCR	bird	9	9	0	0
Tularaemia	<i>Francisella tularensis</i>	Cult	monkey	8	6	0	2
		Cult	beaver	2	2	0	0
		Cult	hare	10	6	0	4
Blackleg	<i>Clostridium chauvoei</i>	IF	cattle	9	7	2	0
		Cult		9	7	0	2
Coxiellosis	<i>Coxiella burnetii</i>	Micr	cattle	179	174	5	0
		ELISA		50	44	0	6
		PCR		82	82	0	0
		Micr	sheep	7	6	1	0
		ELISA		1	1	0	0
		PCR		4	4	0	0
		Micr	goat	6	5	1	0
		ELISA		2	2	0	0
	PCR		4	4	0	0	

3.2. Antimicrobial Resistance Monitoring

Programme concerning Swiss food producing animals

Cloacal swabs from broiler herds were collected at slaughter and cultured for *E. coli*, *Enterococcus* spp., *Campylobacter* spp. and Extended Spectrum beta-Lactamases (ESBLs) producing *E. coli*. Moreover fresh chicken meat from retails was analysed for ESBLs and Methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore nasal swabs from pigs were tested for Methicillin-resistant *S. aureus* (MRSA). Isolated strains and all *Salmonella* spp. strains from diagnostics and reference function were

tested for antimicrobial susceptibility. For testing the Minimal Inhibitory Concentration (MIC) technique by broth microdilution was performed. Results of the antimicrobial resistance monitoring will be published in the ARCH-Vet Report in cooperation of the FVO with Swissmedic.

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

Animal	number of analyses
pigs	301
Fresh chicken meat	319
Total	620

Number of analyses on ESBL producing *Enterobacteriaceae*

Animal	number of analyses
broiler	300
Fresh chicken meat	319
Total	619

Number of analyses on *E. coli*

Animal	number of analyses
broiler	205
Total	205

Number of analyses on *Enterococcus* spp.

Animal	number of analyses
broiler	354
Total	354

Number of analyses on *Campylobacter* spp.

Animal	number of analyses
broiler	496
Total	496

3.3. Reference Activity for Epizootics (Notifiable Animal Diseases)

Confirmation of results for other laboratories

Epizootic	Method	Animal	Total	negative	suspi- cious	positive
Bovine brucellosis	ELISA	cattle	6	3	1	2
	RBT	cattle	6	4	0	2
	CFT	cattle	6	5	0	1
Caprine and ovine brucellosis	ELISA	sheep/ goat	2	1	0	1
	RBT	sheep	2	2	0	0
	CFT	sheep	2	2	0	
Ovine epididymitis	ELISA	sheep	0	0	0	0
Contagious equine metritis	PCR	horse	0	0	0	0
	Cult		0	0	0	0
Blackleg	Cult	cattle	0	0	0	0
	PCR		0	0	0	0
Enzootic pneumonia in swine	PCR lung	swine	1	1	0	0
Campylobacteriosis <small>*1 x <i>C. fetus</i> subsp. <i>fetus</i></small>	ID	dog	125	22	2	101
		cat	18	2	0	16
		cattle	31	2	4	25*
		other	8	2	1	4
Yersiniosis	ID	cattle	0	0	0	0
	ID	fish	3	0	0	3
Multidrug resistance	ID, MIC	diverse	36	0	0	36

Serotyping of *Salmonella* sp. from other laboratories

Serovar	Animal	Number
S. Abortusovis	sheep	1
S. Agona	environment	1
S. Albany	chicken	5
S. Blukwa	snake	1
S. Braenderup	chicken	1

Serovar	Animal	Number
S. Chester	chicken	2
S. Enteritidis	cattle	4
	chicken	13
	snake	2
<i>S. enterica</i> subsp. <i>arizonae</i> 56: z4,z23: - *	snake	1
<i>S. enterica</i> subsp. <i>diarizonae</i> 61: k: 1,5,7	sheep	4
	unknown	3
<i>S. enterica</i> subsp. <i>diarizonae</i> 61: -: 1,5,7	sheep	1
<i>S. enterica</i> subsp. <i>enterica</i> 13,23: i :- *	chicken	5
<i>S. enterica</i> subsp. <i>enterica</i> rauh: d :1,2 *	chicken	1
<i>S. enterica</i> subsp. <i>enterica</i> rauh: i :1,5*	chicken	1
<i>S. enterica</i> subsp. <i>enterica</i> 47:z4,z23:- *	cattle	1
<i>S. enterica</i> subsp. <i>houtenae</i> 38 : z4,z23 - *	snake	1
<i>S. enterica</i> subsp. <i>houtenae</i> 50 g,z51 - *	saurian	1
S. Idikan	chicken	5
S. Indiana	chicken	1
	turkey	2
S. Kisaware	saurian	1
S. Lexington	Chicken	1
S. Matopeni	snake	1
S. Mbandaka	chicken	2
S. Muenchen	snake	1
S. Newport	dog	1
	snake	1
S. Panama	swine	1
S. Rissen	swine	1
S. Schwarzengrund	chicken	3
S. Senftenberg	chicken	2
S. Telelkebir	tortoise	1
S. Tennessee	chicken	1
S. Typhimurium	cattle	11
	chicken	13
	dog	1
	environment	2
	goose	1
	horse	1
	parrot	1
	pigeon	4
	sheep	1

Serovar	Animal	Number
S. Typhimurium, monophasic variant (4,12 : i : -)	cattle	19
	chicken	2
	swine	1
S. Veneziana	cat	1
S. Welikate	chicken	1
No salmonella		10
total		142

* serotyping in human reference laboratory

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

Biovar I	apx group: I BD + II CA	7,12	48
Biovar I	apx group: I BD + II CA + III CA + BD <i>cps2</i> gene positive	2	13
Biovar I	apx group: I CA + BD	10	1
Biovar I	apx group: II CA + III CA + BD	3	11
Biovar I	apx group: III CA + BD	3 variant	10
Biovar II	apx group: I BD + II CA <i>cps2</i> gene positive	2	5
No APP	-	-	11
		Total	99

3.4. Diagnostic Activity for the Border Veterinary Control

Seafood	Total	negative or <100 cfu/g	positive or >100 cfu/g
Detection of <i>Salmonella</i> spp.	17	17	0
Enumeration of <i>L. monocytogenes</i>	21	21	0

Beef	Total	negative	positive
Detection of <i>Salmonella</i> spp.	31	31	31
Detection of VTEC	31	30	1

3.5. Research, Development and Validation

3.5.1. Leptospirosis in a foal – Diagnosis by Real-time PCR

Manuela Stettler, Isabelle Brodard and Sabrina Rodriguez-Campos in collaboration with the horse Clinic at the Vetsuisse Faculty Bern

Real-time PCR is a rapid method for the detection of pathogenic *Leptospira* sp. in urine. However, the microscopic agglutination test (MAT) remains irreplaceable for the detection of the causing serovar. Leptospirosis is caused by pathogenic serovars of the spirochete *Leptospira* sp. The disease occurs worldwide and can affect a broad range of animal species including humans. The clinical signs are variable, ranging from flu-like symptoms to multiple organ failure. *Leptospira* sp. can also lead to abortion and weak offspring. We present a case of leptospirosis in a newborn foal which was detected by Real time PCR from urine as alternative to the serological analysis by MAT.

Publication: Stettler M., Fouché N., Graubner C., Brodard I., Rodriguez-Campos S. Leptospirosis in a foal – Diagnosis by Real-time PCR “9th Annual Meeting of the Swiss Equine Research Network (10th April 2014, Avenches). Schweizer Archiv für Tierheilkunde. Band 156, Heft 4, S. 193, April 2014.

3.5.2. Antimicrobial susceptibility of *Trueperella pyogenes*

Isabelle Bouissou and Gudrun Overesch

Trueperella pyogenes, previously known as *Arcanobacterium pyogenes*, is a gram positive suppurative agent causing notably mastitis in cattle, and other diseases such as abscesses and pneumonia in further species, especially cloven-hoofed animals. The purpose of this study was to determine and compare the antimicrobial susceptibility of 79 *Trueperella pyogenes* isolates from various species against 18 antibacterial drugs often used in veterinary medicine. Minimal inhibitory concentrations (MIC) were determined using the broth microdilution method in a cation-adjusted Mueller-Hinton broth. Elevated MICs were found in 55 isolates against tetracycline (69.6%) and in 9 isolates against macrolides (11.4%). Regarding the 57 strains isolated exclusively from adult cattle, rate of elevated MICs against tetracycline was 80.7%. Comparatively, drugs of the beta-lactam group were found to remain effective against *T. pyogenes*. Except for trimethoprim/sulfamethoxazole, were one isolate showed a distinctly elevated MIC, no indication of resistance against the other antibacterial drugs (cephalosporins, fluoroquinolones and amphenicols) was found.

Publication: Master thesis at the Vetsuisse Faculty of Bern, 2013

3.5.3. Outbreak of Morel's disease in a Swiss goat flock

Bigna Rossetti and Gudrun Overesch in collaboration with the Tierarztpraxis Calanda, Chur, Graubünden and the Office of Food Safety and Animal Health, Chur, Graubünden

Staphylococcus aureus subsp. *anaerobius* is the causative agent of a disease in goats and sheep known as abscess disease or Morel's disease. The main clinical features of affected animals are abscesses in the superficial lymph nodes of the mandibular, superficial cervical, subiliac and popliteal regions, among others (de la Fuente and Suarez 1985, de la Fuente and others 2011). Morel's disease was described for the first time in 1911 in France. Since the initial discovery, the disease has been detected in other European countries, including Spain, Poland, Italy and Croatia, and has also been described in Africa and Asia (de la Fuente and others 2011). To our knowledge, it has not been diagnosed in Switzerland to date. Herein, we describe the first thoroughly analyzed outbreak of Morel's disease in a Swiss goat flock. All of the Swiss isolates have been shown to be sensitive to all tested antibiotics in vitro. However, antibiotic treatment of abscesses is often difficult due to encapsulation of the pathogen. Other measures, such as surgical excision of abscesses, separation of infected animals or reduced crowding in the barns, were taken to control spread of the disease (Gezon and others 1991). After diagnosis of Morel's disease, all 14 affected goats were slaughtered. Morel's disease is infectious and is rapidly transmitted from goat to goat; successful treatment is not guaranteed. Therefore, early detection and correct identification of new infections is essential for a reduction in infection risk. Helpful measures for Morel's disease prevention include regular and careful clinical examination of all goats and sheep, especially after returning from the Alp or after contact with goats from other flocks in general.

Publication: Rossetti B, Regi G., Röttele K., Overesch G., Outbreak of Morel's disease in a Swiss goat flock. Vet Rec Case Rep 2014; 2:1

5 Publications

5.1. Peer-Reviewed Publications

Publication List

Amar Ch., Kittl S., Spreng D., Thomann A., Korczak B. M., Burnens A., Kuhnert P. (2014) Genotypes and antibiotic resistance of canine *Campylobacter jejuni* isolates. *Vet Micro* 168: 12-130. DOI: 10.1016/j.vetmic.2013.10.006

Bernasconi Ch, Bodmer M, Doherr MG, Janett F, Thomann A, Spycher C, Iten C, Hentrich B, Gottstein B, Müller N, Frey CF (2014) *Tritrichomonas foetus*: prevalence study in naturally mating bulls in Switzerland. *Vet Parasitol.* 200:289-94. DOI: 10.1016/j.vetpar.2013.12.029

Berset-Istratescu C. M., Glardon O.J., Magouras I., Frey C.F., Gobeli S., Burgener I.A. (2014) Follow-up of 100 dogs with acute diarrhoea in a primary care practice. *The Veterinary Journal* 199: 188–190. DOI: 10.1016/j.tvjl.2013.10.014.

Bianchini, V., Luini, M., Borella, L., Parisi, A., Jonas, R., Kittl, S., Kuhnert, P. (2014) Genotypes and antibiotic resistances of *Campylobacter jejuni* isolates from cattle and pigeons in dairy farms. *Int.J. Environ. Res. Public Health* 11:7154-7162. DOI:10.3390/ijerph110707154

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Christensen, H., Kuhnert, P., Norskov-Lauritsen, N., Planet, P.J., Bisgaard, M. *Pasteurellaceae*. In: *The Prokaryotes, Gammaproteobacteria*. 4th Edition. Edited by Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F. Springer-Verlag Berlin Heidelberg, 2014. DOI 10.1007/978-3-642-38922-1_224

Chanchaithong, P., Perreten V., Schwendener S., Tribuddharat C., Chongthaleong A., Niyomtham W., and Prapasarakul N.. 2014. Strain typing and antimicrobial susceptibility of methicillin-resistant coagulase-positive staphylococcal species in dogs and people associated with dogs in Thailand. *J. Appl. Microbiol.* 117(2):572-586. DOI: 10.1111/jam.12545.

Couto, N., Belas A., Couto I., Perreten V., and Pomba C. 2014. Genetic relatedness, antimicrobial and biocide susceptibility comparative analysis of methicillin-resistant and -susceptible *Staphylococcus pseudintermedius* from Portugal. *Microb. Drug Resist.* 20(4):364-371. DOI: 10.1089/mdr.2013.0043.

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Falquet L, Liljander A, Schieck E, Gluecks I, Frey J, Jores J. (2014) Complete genome sequences of virulent *Mycoplasma capricolum* subsp. *capripneumoniae* strains F38

and ILRI181. Genome Announc. Oct 16;2(5). pii: e01041-14. DOI: 10.1128/genomeA.01041-14.

Frey, J. and Falquet, L. (2014) Patho-genetics of *Clostridium chauvoei*. Research in Microbiology 11:0923. DOI: 10.1016/j.resmic.2014.10.013

Gormley E, Corner LAL, Costello E, Rodriguez-Campos S. (2014) Bacteriological diagnosis and molecular strain typing of *Mycobacterium bovis* and *Mycobacterium caprae*. Research in Veterinary Science 97:S5–S19 DOI: 10.1016/j.rvsc.2014.04.010

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Lupo A., Vogt D., Seiffert S.N., Endimiani A., and Perreten V. 2014. Antibiotic resistance and phylogenetic characterization of *Acinetobacter baumannii* strains isolated from commercial raw meat in Switzerland. J. Food Prot. 77(11):1976-1981. DOI: 10.4315/0362-028X.JFP-14-073.

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effects in the attachment protein of canine distemper virus. *J Virol.* Volume 88, (14):8057-64. DOI: 10.1128/JVI.00454-14.

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7. Organization chart (Organigramm)



