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KEYNOTE TALKS

K-01 *Chlamydia trachomatis* effector Dre1 interacts with dynactin to reposition host organelles during infection

Jessica Sherry [1], Lee Dolat [2], Eleanor McMahon [1], Danielle L. Swaney [3], Robert J. Bastidas [2], Jeffrey R. Johnson [3], Raphael H. Valdivia [2], Nevan J. Krogan [3], Cherilyn A. Elwell [1], *Joanne N. Engel* [1,4]

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Chlamydia trachomatis is an obligate intracellular pathogen that replicates within a specialized membrane-bound compartment, called the inclusion. *Chlamydia* species express a unique class of effectors, Incs, which are translocated from the bacteria by a Type III secretion system and are inserted into the inclusion membrane where they modulate the host-bacterium interface. *C. trachomatis* repositions specific host organelles during infection to acquire nutrients and evade host cell surveillance, however the bacterial and host proteins controlling these processes are largely unknown. Here, we identify an interaction between the host dynactin complex and the *C. trachomatis* Inc CT192 (CTL0444), hereafter named Dre1 for Dynactin Recruiting Effector 1. We show that dynactin is recruited to the inclusion in a Dre1-dependent manner and that loss of Dre1 diminishes the recruitment of specific host organelles, including the centrosome, mitotic spindle, primary cilium, and Golgi apparatus to the inclusion. Inactivation of Dre1 results in decreased *C. trachomatis* fitness in cell-based assays and in a mouse model of infection. By targeting particular functions of the versatile host dynactin complex, Dre1 facilitates re-arrangement of certain organelles around the growing inclusion. Our work highlights how *C. trachomatis* employs a single effector to evoke specific, large-scale changes in host cell organization that establish an intracellular replicative niche without globally inhibiting host cellular function.

K02 Genomics of Rickettsia species unveils an unsuspected level of genetic exchange

Pierre-Edouard Fournier **[1,2,3], Khalid El Karkouri [1,2], Linda Houhamdi [2,3], Didier Raoult [2,3,4]**

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Rickettsia species are endosymbiotic bacteria living in association with various arthropods and able to cause in humans a variety of mild to fatal diseases. Being strictly intracellular, in humans they infect endothelial cells and undergo a progressive genomic reduction. However, some *Rickettsia* species have been demonstrated to survive and multiply in amoebae where they might, as in arthropods, be in contact with other microorganisms. Therefore, although their lifestyle suggested that they may be protected from genetic exchanges, the presence of plasmids in 19 species led us to assume otherwise. By analysing the evolution and diversity of 34 *Rickettsia* species using a pangenomic meta-analysis, we observed that *Rickettsia* spp. diverged into two Spotted Fever groups, a Typhus group, a Canadensis group and a Bellii group, and may have inherited their plasmids from an ancestral plasmid that persisted in some strains or may have been lost by others. We also observed that the *Rickettsia* genus evolved through a strong interplay between genome degradation/reduction and/or expansion leading to possible distinct adaptive trajectories. *Rickettsia* species mainly exchanged genetic material with α-proteobacteria, and also with γ/β/δ-proteobacteria, cytophagia, actinobacteria, cyanobacteria, chlamydiia and viruses, suggesting lateral transfer of several critical genes. These exchanges, even with diverse distant lineages, have probably been promoted by the presence of abundant mobile genetic elements, especially in the spotted Fever and Bellii groups. Therefore, it appears that during their diversification and adaptation to eukaryotic hosts, rickettsial genomes may have been shaped by diverse evolutionary processes.

K-03 *Anaplasma phagocytophilum* Host-Pathogen Interactions: New Insights into Mechanisms For Host Cell Invasion, Intracellular Survival, and Dissemination

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Anaplasma phagocytophilum is a tick-transmitted obligate intracellular bacterium that causes granulocytic anaplasmosis, a potentially severe infection of humans and some domestic animals and an emerging global health threat. Treatment options are limited, and no vaccine exists. Its evolution as an endosymbiont that commandeers granulocytes to complete its enzootic cycle engenders *A. phagocytophilum* success as a pathogen when vectored to accidental human and other non-reservoir hosts. Understanding the mechanisms by which it invades, propagates within, and disseminates from host cells could yield new targets for preventing or treating disease. This keynote will describe how *A. phagocytophilum* uses multiple adhesins to build a signaling platform that facilitates entry into its host cell-derived vacuole and how interfering with one or more of these interactions impairs infection. The *A. phagocytophilum* vacuole (ApV) is a hub that acquires vesicular traffic from numerous organelle sources to drive its intracellular parasitism and infectious progeny production. The underlying mechanism by which it does so has remained poorly understood. The lecture will also mechanistically explain how the ApV promiscuously interacts with multiple membrane trafficking pathways as well as how it can be targeted to impede different infection cycle stages. Overall, this talk will review new insights into *A. phagocytophilum* molecular pathogenesis and potential novel targets for therapeutic intervention.

MAIN & SHORT TALKS

M-01 **Fine-tuning host cholesterol in the bacterial intracellular niche**

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The obligate intracellular bacterial pathogen *Coxiella* replicates in a fusogenic phagolysosome-like vacuole called the *Coxiella*-containing vacuole (CCV). While host cholesterol readily traffics to the CCV, *Coxiella* is exquisitely sensitive to cholesterol accumulation in the CCV membrane, with elevated cholesterol leading to increased CCV acidification, proteolytic activity, and bacterial death. Thus, bacterial regulation of CCV cholesterol content is essential for *Coxiella* survival and pathogenesis. *Coxiella* utilizes multiple approaches to regulate CCV cholesterol, including membrane contact sites between the CCV and ER, modulating expression of host cholesterol metabolic genes, and increasing cholesterol storage in lipid droplets. Most recently, we discovered that *Coxiella* expresses a sterol modifying enzyme, Stmp1 (CBU1206). Stmp1 has homology to eukaryotic sterol reductases but does not synthesize cholesterol, indicating it may have a unique function during *Coxiella* infection. While Stmp1 is not essential for axenic growth, intracellular Stmp1 knockout bacteria form smaller CCVs that accumulate cholesterol and are more proteolytically active, correlating with a significant intracellular growth defect in both epithelial cells and macrophages. Wildtype and Stmp1 KO bacteria grew similarly in cholesterol-free cells, but the Stmp1 KO was hypersensitive to cholesterol supplementation, further supporting a link between cholesterol and Stmp1. Using a live cell trafficking assay and endosomal markers, we determined that the Stmp1 KO CCVs are as fusogenic as wildtype CCVs but preferentially fuse with host lysosomes and are more acidic than wildtype CCVs. To better understand the underlying mechanism behind the Stmp1 KO defect, we performed sterol profiling. Surprisingly, we found that cells infected with the Stmp1 KO accumulated the potent cholesterol homeostasis regulator 25-hydroxycholesterol (25-HC). Further, 25-HC increases CCV proteolytic activity and inhibited growth of wildtype bacteria. Collectively, these data indicate that CBU1206 alters host cholesterol metabolism and is essential for *Coxiella* growth in host cells. Further, the host oxysterol 25-HC functions in host defense against *Coxiella* infection.

M-02 ***Anaplasma*: cell corruption by an intracellular bacteria**

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Anaplasma spp. have increased in number, niches, and in the diversity of roles they play as either endosymbionts or pathogens that induced acute and or chronic disease. To date, human infection by only 3 species has been definitively identified, including *A. phagocytophilum*, *A. capra*, and *A. ovis.* Although, identified as *Cytoecetes microti* and *Rickettsia phagocytophila* over 100 years ago, *A. phagocytophilum* likely exists in a range of clades with distinct infection propensities and varying biological properties. The focus of this work will examine the range of biological properties of *A. phagocytophilum* infection in mammalian cells, predominantly neutrophils, some of the key cellular perturbations and their mechanisms, and also the key relationships that lead to "corruption" of cellular and organismal functions that underlie disease. Key areas of focus will include mechanisms by which *A. phagocytophilum* adheres to and enters cells, key molecular determinants of those processes and its colonization of neutrophil vacuoles. Additional discussion will include the role of the moonlighting cytoplasmic effector and nucleomodulin AnkA, as well as potential roles for other nucleomodulins. How these forces integrate to manipulate the neutrophil will be examined, with an additional focus on interactions with other host immune cells that influence immune response and its evasion. The major goals is to examine the resulting mechanisms of disease and their potential use as targets for preventive and therapeutic approaches.

S-01* Alternative splicing variant of mixed lineage leukemia 5 (vMML5), a novel cellular receptor for Orientia tsutsugamushi

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Background

Scrub typhus is a mite-borne disease caused by *Orientia tsutsugamushi* infection. It is obligatory for intracellular gram-negative bacteria to be internalized into host cells for replication; however, the precise mechanism of intracellular invasion of *O. tsutsugamushi* has been poorly characterized. Previous studies have demonstrated that *O.tsutsugamushi* encodes six different numbers of autotransporter genes (*scaA–scaF*), of which ScaA is present in diverse genotypes and involved in the adherence of host cells but the interacting host receptor is yet to be elucidated.

Method

In this study, using yeast two-hybrid (Y2H) screening, we identified a mixed-lineage leukemia 5 (MLL5) mammalian trithorax group protein as a host receptor that interacts with ScaA. The identified MLL5 was an alternative splicing variant including transmembrane domain. The vMLL5 is expressed in plasma membrane and intracellular compartments.

Results

ScaA-expressing Escherichia coli showed a significantly increased adherence to vMLL5-overexpressing cells when compared with control cells. We mapped the interaction motives in vMML5 receptor and ScaA ligand of *O. tsutsugamushi*. Immunization of the vMLL5-binding passenger fragment of ScaA provided superior protection against lethal challenge than non-binding fragment.

Conclusions

These results suggest that vMLL5 is a novel host receptor for *O. tsutsugamushi* infection, and the vMLL5-binding domain of ScaA could be used as a promising vaccine candidate against scrub typhus.

*Student paper

S-02 Characterization of a novel *Anaplasma phagocytophilum* effector involved in tick infection

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Background
A number of intracellular pathogens have a Type IV Secretion System (T4SS) that delivers effector molecules into the host cell cytosol. Although the T4SS machinery is relatively easy to identify, the proteins that it translocates are not. Much effort has been expended to identify effectors that interact with the mammalian host, but relatively little to examine those that specifically interact with a transmission vector. In this work we analyze an effector for *Anaplasma phagocytophilum* (Ap) that appears to be tick specific.

Method
We used the OPT4e program to predict Ap effectors and tested them for translocation using a heterologous *Legionella pneumophila* translocation assay. Transcription in mammalian and tick cell lines was analyzed by qPCR, and growth assays in mammalian and tick cells of wild type (WT) and a transposon knockout mutant (Tn) were performed. The WT and Tn lines were used to infect mice, larval ticks were fed on the mice and infection burden was assayed in mice and ticks. Ectopically expressed GFP-fusion protein in HeLa was visualized and localized using fluorescent markers/antibodies.

Results
One of the predicted effectors was shown to be translocated in a T4-dependent manner. It displayed a tick specific expression pattern, and a knock-out Tn cell line had no growth defect in mammalian cells, but did not grow in tick cells. The Tn mutant colonized mice to similar levels as WT, but exhibited a deficit when colonizing tick larvae. Ectopic expression of GFP-tagged fusion protein showed localization with actin and altered cytoskeleton morphology. Truncation mutants identified separate regions responsible for actin co-localization, actin remodeling, and plasma membrane association. Bioinformatic analysis indicates that the protein may be phosphorylated.

Conclusions
In this work we have identified and characterized an effector from Ap that appears to be important for colonization and intracellular growth in the tick vector.

M-03 ***Mycoplasma pneumoniae* – clinical epidemiology**

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Mycoplasma pneumoniae is a leading pathogen causing respiratory infections. Few of the known epidemiological features, include an «Olympic» epidemic pattern of surge in infections every few years, infections in pediatric population, and in elderly, and the ability to cause infections both in pediatric and in elderly populations. While considered by many to be a pathogen with low virulence causing mainly mild diseases, we as well as others have suggested that there is also severe presentation leading both ICU admission and mortality. *M. pneumoniae* is transmitted via aerosols, and spread is documented in close communities, families and army recruits, where it causes outbreaks. The ESGMAC have shown recently, the pattern in which some of the epidemic waves spread from north to south. Nevertheless, many questions regarding this the epidemiology of this pathogen are still open.

Since late 2019 the SARS-COV-2 virus caused a global pandemic. One of the main impact of the pandemic was the use of nonpharmaceutical interventions such as social distancing, the use of masks, lockdowns and stay-at-home orders, school closures, and travel restrictions. An interesting outcome was a major impact on other respiratory pathogens such as Influenza and RSV. Additionally, such measures had an affect also other respiratory pathogens such as *M. pneumoniae*. It this review lecture I will address recent findings on *M. pneumoniae* epidemiology in the context of the pandemic and the associated effect on the number of reported cases of this unique wall less bacterial pathogen.

M-04 ***Mycoplasma pneumoniae* infections: genotypes, outcome, and COVID-19**

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Mycoplasma pneumoniae is a major bacterial cause of respiratory tract infections in children and adults. *Mycoplasma pneumoniae* is transmitted by aerosol and droplets in close contact. Although the infection is generally mild, patients of every age can develop severe and fulminant disease. Apart from the respiratory tract, *M. pneumoniae* can cause a wide range of extrapulmonary manifestations. However, factors leading to the wide range of manifestations associated with *M. pneumoniae* infection are unclear. This talk presents the impact of *M. pneumoniae* genotypes on specific clinical outcomes and the effect of non-pharmaceutical interventions (NPIs) against COVID-19 on *M. pneumoniae* transmission.

Website:
https://www.kispi.uzh.ch/kinderspital/person/meyer-sauteur-phd-patrick-m

Link to publications relevant to the talk:
DOI: 10.1128/JCM.00748-21
DOI: 10.2807/1560-7917.ES.2022.27.19.2100746

M-05 **Recent Developments in *Mycoplasma***

Vicki Chalker

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Dr Vicki Chalker has worked on Mycoplasma for more than 20 years, first at the Royal Veterinary College and at the UK Health Security Agency. Mycoplasma are fascinating small bacteria that infect a range of organisms including humans. They have economic, medical and veterinary importance. Recent advances in human mycoplasma will be discussed, including Mycoplasma pneumoniae, Ureaplasma and other aspects.

In addition taxonomy will be discussed. The International Committee on Systematics of Prokaryotes (ICSP) oversees the nomenclature of prokaryotes, naming rules and issues Judicial Commission Opinions concerning taxonomy, Bacteriological Code revisions, etc. Mycoplasmology is represented by a subcommittee of experts on behalf of the International Organization for Mycoplasmology (IOM). Taxonomy of Mollicutes standards (Brown et al., 2007) include deposition: (i) type strains into two culture collections; (ii) 16S rRNA gene sequences with phylogenetic analysis; (iii) demonstration type strains differ from known valid species; (iv) assignment to an order, family and genus in the class. (v) description of novel Mollicutes species should also follow the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes in which genome sequence (complete or draft) the proposed type strain is required (this is not required for `Candidatus` assignment) (Chun et al., 2018). Antiserum is no longer required (Firrao and Brown 2012). Submission of Mollicutes to culture collections is imperative to assist the community longer term by accessing strains, retaining them for future work and is required when describing a new species. In 2021 the ICSP reviewed international culture collections accepting depositions of Mollicutes. The following collections indicated they accept strains (*with no charge for deposition, shipping fees apply): USA: ATCC* France: CIP* Germany: DSMZ* Japan: NBRC UK: NCTC* Taiwan: BCRC (case by case review) Taxonomic naming of new species: Recent publications suggest renaming of species to unusual/unconventional names (Gupta and Oren, 2020). The ICSP Mollicutes Subcommittee is committed to retaining nomenclature aligned to the agreed code and existing scientific/clinical practice, retaining standards for authors naming Mollicutes. Balish et al., 2020 recommended rejection of published names within the class Mollicutes. When considering naming new species, we recommend contacting the committee for advice prior to publication, engage with the Mollicute community prior to publication of controversial changes, especially impacting pathogenic relevant bacteria.

M-06 **Diagnosis of intracellular bacteria by PCR**

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Cultivation of intracellular bacteria are challenging and comprehensive. Same-day detection of these bacteria may contribute to rapid and targeted treatment. Polymerase chain detection (PCR) is a fast and accurate method for detection. An update on current protocols for PCR detection of selected intracellular bacteria will be presented.

M-07 What's new in chronic Q fever and Q fever fatigue syndrome?

Chantal Bleeker-Rovers

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More than ten years after the largest Q fever outbreak that ever occurred ended in the Netherlands, a lot has been learned about this infection. After infection 1-5% of people develop chronic Q fever, most often presenting as endocarditis or vascular infection. Chronic Q fever is difficult to diagnose, difficult to treat and often leads to serious complications with high mortality. Around 20% of people develop Q fever fatigue syndrome after acute Q fever, a debilitating disease of still unknown cause. In this talk the newest results from the Dutch National Chronic Q fever database will be presented with new data on the long possible time between acute Q fever and diagnosis of chronic Q fever, the diagnostic value of FISH for diagnosis and of serology for follow-up, and prognosis. Also, results on treatment and outcome of vascular chronic fever will be discussed in more detail. More insight into the possible immunological etiology of Q fever fatigue syndrome will be given as well as un update on the long-term effect of cognitive behavioral therapy.

S-03* Nanomotion-based antimicrobial susceptibility test: an innovative method to study intracellular bacteria such as Chlamydia trachomatis and Waddlia chondrophila

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Background

Chlamydial antibiotic resistance has become more common throughout recent years. However, antimicrobial susceptibility test (AST) is rarely used in chlamydia diagnosis, as it requires cell-culture, which is technically challenging and costly. Therefore, there is a need to develop easy, rapid and reliable AST for chlamydia and other fastidious intracellular bacteria. For extracellular bacteria, like *E. coli*, it has previously been shown that nanomotion-based AST reduces the time to results compared to standard AST methods. By using micromechanical cantilever-based sensors, natural nanomotion of living bacteria is recorded and sensitivity or resistance to antibiotics can be measured within minutes to hours. Nanomotion-based AST has the potential to create an entirely new segment in microbiological diagnostics for strict intracellular bacteria such as *Chlamydiales.*

Method

In this study, we aimed to use a technology relying on micromechanical cantilever-based sensors to measure the nanomotion of *Chlamydiales* elementary bodies (EBs), the extracellular non-growing forms of the bacteria. We attached EBs of *C. trachomatis*, the most frequent sexually transmitted bacterial pathogen in Western Europe and the US, and of *W. chondrophila,* a *Chlamydia*-related bacterium emerging as an agent of miscarriage, to the micromechanical sensor and measured their nanomotion.

Results

We optimized attachment of *C. trachomatis* and *W. chondrophila* EBs to micromechanical sensor, by testing several linking agents. We also demonstrated that the specific nanomotion signal was strongly diminished upon chemical inactivation of bacteria, such as paraformaldehyde fixation.

Conclusions

Within this project, we demonstrated that nanomotion of chlamydial EBs can be detected with this technology. Further development of this method is now required in order to achieve a culture-independent measurement of viability and/or infectivity of chlamydial EBs. In addition, this method could be applied to other strict intracellular such as *Rickettsia*, responsible of the spotted fever and typhus, or *Coxiella*, the causative agent of Q fever.

*Student paper

S-04 Fluorescence in situ hybridization for detecting Coxiella burnetii in tissue samples from chronic Q fever patients

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Background

Detection of the intracellular bacterium *Coxiella burnetii*, causative agent of chronic Q fever, is notoriously difficult. Diagnosis of and duration of antibiotic treatment for chronic Q fever is determined by detection of the bacterium with polymerase chain reaction (PCR). Fluorescence *in situ* hybridization (FISH) might be a promising technique for detecting *C. burnetii* in tissue samples from chronic Q fever patients, but its value in comparison with PCR is uncertain.

Method

FISH and PCR were performed on tissue samples from Dutch chronic Q fever patients collected during surgery or autopsy. Sensitivity, specificity, and overall diagnostic accuracy were calculated. Additionally, data on patient and disease characteristics were collected from electronic medical records.

Results

Of 39 chronic Q fever patients, 49 tissue samples from mainly vascular walls, heart valves, or placentas were examined by FISH and PCR. The sensitivity and specificity of FISH compared to PCR for detecting *C. burnetii* in tissue samples from chronic Q fever patients was respectively 45.1% and 85.7%. The overall diagnostic accuracy was 57.8% (95% CI, 42.2% - 72.3%). Two *C. burnetii* PCR negative placentas were FISH positive. Four FISH results (8.2%) were deemed inconclusive because of autofluorescence.

Conclusions

With an overall diagnostic accuracy of 57.8%, we cannot recommend the replacement of PCR by FISH in the routine diagnostics of chronic Q fever.

M-08 **Effects of *Chlamydia trachomatis* infection on the centrosome and primary cilia**

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Background

During their biphasic developmental cycle, bacteria of the genus *Chlamydia* have many interactions with their eukaryotic host cell, including effects on the centrosome, the major cellular microtubule organizing center. *Chlamydia* cause centrosome amplification, a hallmark of cancer. This bacterium is a proposed co-factor for HPV in the development of cervical cancer, but it is not known if *Chlamydia's* contributions to HPV-driven carcinogenesis is through effects on the centrosome. It is also not clear if *Chlamydia* affects primary cilia, centrosome-derived signaling organelles that are present on quiescent cells and which are also linked to cancer.

Methods

We have addressed these questions with two specialized tissue culture systems. 1) We modelled HPV/*Chlamydia* co-infection by performing *Chlamydia* infection in cells expressing E6 and E7, two HPV oncogenes that are sufficient to induce centrosome amplification. 2) We infected ciliated cells in which ciliogenesis was experimentally induced. Before or after infection, these tissue culture systems were subjected to pharmacological and genetic manipulations, and effects on the centrosome, cilia and infectious progeny were determined.

Result

We found that *C. trachomatis* caused centrosome amplification in a greater proportion of cells than HPV and that the effects of the two pathogens on the centrosome were additive. Furthermore, *Chlamydia* and HPV induced centrosome abnormalities through different mechanisms, with the chlamydial effect being largely due disruption of cytokinesis. We also observed that *C.trachomatis* infection induced primary cilia disassembly through an AurA and HDAC6-dependent pathway. Blocking Chlamydia-induced cilia disassembly disrupted infectious progeny production.

Conclusions

We propose that the additive effects of *Chlamydia* and HPV on centrosome number provide biological plausibility for *Chlamydia* as co-factor for HPV in the development of cervical cancer. Furthermore, our results indicate that cilia disassembly and/or the cilia regulatory pathway are critical for the infection, although how cilia are linked to progeny production is unclear.

M-09 **The Type Three Secretion System of *W. chondrophila***

Carole Kebbi-Beghdadi, Ludovic Pilloux and Gilbert Greub

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Waddlia chondrophila is an emerging pathogen, phylogenetically related to *Chlamydiae* and causing adverse pregnancy outcomes in humans and abortion in ruminants. As an obligate intracellular bacterium, *W. chondrophila* needs to modulate its environment to establish a replicative niche, acquire nutrients and escape the host defense mechanisms. These bacteria-host interactions are triggered by effector proteins secreted mainly by the Type 3 Secretion System (T3SS). This secretion system is a syringe-like structure spanning the inner and outer bacterial membranes as well as the inclusion membrane. Its structural proteins as well as the chaperones required to maintain effectors in a secretion-competent state are very well conserved between all known members of the *Chlamydiales* order. On the opposite, effectors are poorly conserved and largely species-specific. Indeed, only a few T3SS effectors identified in *Chlamydiae* have homologs in *W. chondrophila,* which probably reflects the very different life styles of these bacteria.
We used several approaches to identify *W. chondrophila* putative T3SS effectors, some relying on bioinformatics tools and algorithms, other based on mass spectrometry analyses of proteins present in the cytosol of *W. chondrophila*-infected cells or of proteins interacting with T3SS chaperones. In addition, as *W. chondrophila* is not amenable to genetic modifications, we confirmed Type 3-dependent secretion of these newly identified effectors using a heterologous expression system, *Yersinia enterocolitica.*
With these diverse approaches, we identified several novel *W. chondrophila* effectors, including one protein localized in the inclusion membrane (Inc) and one protein targeted to the host cell nucleus upon infection and showing a high affinity for chromatin. Their gene and protein expression profiles as well as their subcellular localization during the course of a *W. chondrophila* infection were characterized.

S-05 The role of 24 SNPs in host genes and the development of long-term Chlamydia trachomatis complications in women

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Background

Host genetics are suggested to explain a large part of the variability in the clinical course of *Chlamydia trachomatis* (CT) infections in women. Using a candidate gene approach, 24 Single Nucleotide Polymorphisms (SNPs) were identified potentially related to severity of CT-infections (figure 1). We aimed to find associations between these SNPs and CT complications in a long-term cohort of women from the Netherlands Chlamydia Cohort Study (NECCST).

Method

Of 5,704 women included in NECCST a selection was made of women with a Western migration background and with a previous CT infection (either CT-PCR+, CT-IgG+ or self-reported CT-infection)(n=884). The outcomes symptomatic CT infection, PID, and tubal factor infertility (TFI)) were questionnaire based. DNA for SNP determination was isolated from buccal swabs, vaginal swabs, or urine. SNP distributions in women with and without complications were compared using $\chi 2$ tests. *Cochran Armitage tests* for *trend* were performed and R2's were calculated for each SNP of interest, assessing the association between SNPs and severity of outcomes.

Results

Among the 884 women included the following outcomes were reported: 40.6% symptomatic CT-infection (359/884), 5.8% PID (51/884), 1.1% TFI (10/884). For women carrying one of the SNPs: TLR2 *rs4696480* T>A (p=0.01), chr21 *rs2298677* A>G (p=0.009) and RXRA *rs9409929* G>A (p=0.03), a slightly higher risk for complications was found. IL10 *rs1800871* T>C carriage was more common in women with PID and TFI (Figure 2). Trends were also observed for NCAM1.2 *rs11214493* T>C; carriage of the heterozygote mutation was increased in women with TFI.

Conclusions

Severity of infection was associated with the following SNPs: IL10 *rs1800871* A>G, IL10 *rs1800896* C>T and NCAM1.2 *rs11214493* T>C. For the TLR2 and TLR9 SNPs also increasing (but non-significant) trends were observed. These results show the potential of SNPs in the identification of women with high risk for CT complications.

Type	Gene	Function	SNP
PRR	TLR2, TLR4, TLR5, TLR9	Toll-like receptors	rs5743708 (G>A), rs4696480 (T>A), rs4986790 (A>G), rs5744168 (C>T), rs187084 (T>C)
	CCR5	Chemokine receptor	rs333 (del)
	NOD1_chr7	Intracellular pattern recognition receptor	rs6958571 (A>C)
Cytokines	IL10_IL19	Anti-inflammatory cytokine	rs1800896 (T>C), rs1800871 (A>G)
	IL1RN	Interleukin-1 receptor antagonist protein	rs419598 (T>C)
	TGFB1	Growth factor beta	rs4803455 (C>A)
Other	chr 21	—	rs2298677 (A>G)
	CYP27B1	Enzyme involved in regulation of vitamin D	rs10877012 (T>C)
	LPAR3	Receptor for lysophosphatidic acid	rs140444859 (G>A), rs373680936 (G>A)
	NCAM1	Natural killer cell markers	rs11214484 (T>C), rs11214493 (T>C), rs3018458 (C>A)
	PREX2	—	rs111513399 (A>G)
	RAB5C	Vesicle transporting protein	rs147419987 (G>A)
	RXRA	Nuclear receptor for retinoic acid	rs9409929 (G>A)
	S1PR3	G-protein-coupled receptor	rs34421923 (G>A)
	VDR	Vitamin D Receptor	rs2228570 (C>T), rs121909792 (C>A)

PRR = pathogen recognition receptors

Figure 1. included snps.png

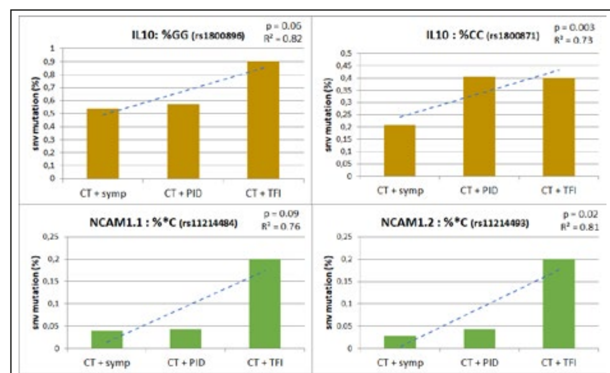

Figure 2. trend analyses.png

S-06 An unexpected wealth of ubiquitin-directed effectors encoded by Chlamydiales

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Background
In Eukaryotes, protein ubiquitination regulates virtually every cellular pathway, including the defense against bacterial pathogens. Many intracellular bacteria secrete ubiquitin-directed effectors into the host cell, which help them to evade ubiquitin-based lysosomal targeting, or even re-directs the ubiquitin system to attack hostencoded defense factors. Typical intracellular bacteria encode a small number of deubiquitinating enzyme (DUBs) and/or ubiquitin ligase effectors. So far, *Legionella pneumophila* is the only known bacterium with a massively expanded and diversified ubiquitin-effector repertoire.

Method
We employed a comprehensive bioinformatical screen to search the genomes of intracellular bacteria for new ubiquitin-directed effectors, based on sequence- and structure-relationships. Based on the screen results, we selected the Chlamydiales species *Simkania negevensis* and *Waddlia chondrophila* for further experimental investigations. We expressed and purified several putative ubiquitin-directed effectors, characterized their function, and solved the structure of several enzyme-substrate complexes.

Results
We identified deubiquitinating enzymes and ubiquitin ligases from *Simkania* and *Waddlia*, including several enzyme classes never before observed in bacteria. *Simkania* encodes at least seven active deubiquitinases with highly unusual specificities, including enzymes selectively cleaving K6-linked or M1-linked ubiquitin chains. Most interestingly, *Simkania* contains a novel enzyme class that is able to cleave ubiquitin chains irreversibly, thereby preventing the re-ubiquitination of the substrate. Both Simkania and Waddlia encode ubiquitin ligases with 'true' HECT-domains, another first sighting in the bacterial world.

Conclusions
We show that the wealth of ubiquitin-directed effectors in the Chlamydiales rivals that of *Legionella pneumophila*. Both *Simkania* and *Legionella* encode K6- and M1-specific deubiquitinases, which are not related to each other. The independent acquisition of those activities by unrelated bacteria with a similar lifestyle highlights the importance of K6- and M1-linked ubiquitin chains in the antibacterial defense.

M-10 **A molecular characterization of *Chlamydia* effectors proteins uncovers new facets of *Chlamydia* pathogenesis**

Raphael H. Valdivia

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Chlamydia trachomatis is responsible for the bulk of sexually transmitted bacterial infections worldwide. *Chlamydia* employs a Type III secretion system to deliver effector proteins that reorganize the cytoskeleton and reprogram host cell signaling to enable invasion, intracellular survival, and replication. However, a molecular understanding of how *Chlamydia* interacts with host cells and tissues remain unknown. We propose that by defining the molecular function of effector proteins, we can better understand the fundamentals of *Chlamydia* pathogenesis. We illustrate this premise by describing the molecular activities of the multifunctional effector TepP (translocated early phosphoprotein) which regulates the Src-dependent tyrosine phosphorylation of EPS8, a filamentous actin-binding protein that localizes to epithelial microvilli, tight junctions (TJs), and endosomes. TepP recruits EPS8 to early *Chlamydia*-containing vacuoles ("inclusions") while simultaneously promoting the disassembly of cell-cell junctions and loss of barrier function in a primary endometrial organoid infection model to enhance invasiveness. The TepP-dependent repurposing of EPS8 to break down epithelial junction is important for pathogenesis as *Eps8-/-* mice are resistant to *Chlamydia* infections. Furthermore we determined that *tepP* mutants are unable to ascend to the upper genital tract and to induced pathology, underscoring the importance of this effector in virulence.

M-11 The *Bartonella* autotransporter CFA is a protective antigen and hypervariable target of neutralizing antibodies blocking erythrocyte infection

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Background

The bacterial genus *Bartonella* comprises several emerging pathogens, which can cause a broad spectrum of clinical manifestations in humans. The targets and mechanisms of anti-*Bartonella* immune defense are ill-defined and bacterial immune evasion strategies remain elusive.

Method

We used the mouse infection model for *Bartonella taylorii* to characterize the immune response to *Bartonella* infection.

Results

We found that experimentally infected mice resolved *Bartonella* infection by mounting antibody responses that neutralized the bacteria, preventing their attachment to erythrocytes and suppressing bacteremia independently of complement or Fc-receptors. *Bartonella*-neutralizing antibody responses were rapidly induced and depended on CD40 signaling but not on affinity maturation. We cloned neutralizing monoclonal antibodies (mAbs) and by mass spectrometry identified the bacterial autotransporter CFA as a neutralizing antibody target. Vaccination against CFA suppressed *Bartonella* bacteremia, validating CFA as protective antigen. We mapped *Bartonella*-neutralizing mAb binding to a domain in CFA that we found is hypervariable in both human- and mouse-pathogenic strains, indicating mutational antibody evasion at the *Bartonella* sub-species level.

Conclusions

These insights into *Bartonella* immunity and immune evasion provide a conceptual framework for vaccine development, identifying important challenges in this endeavor.

S-07 Differential response to environmental stress reveals a transcriptional regulatory pathway, active in Chlamydia trachomatis but not C. muridarum, linking virulence gene expression with persistence

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Background
C. trachomatis genes involved in glycogen metabolism (*glgA*) and virulence (*pgp3*) are down-regulated when chlamydiae infect glucose-limited cells. Expression of OmcA and OmcB, present only in infectious elementary bodies (EB) mark reticulate body (RB) to EB conversion. We determined that *omcAB* transcription is also reduced with restricted glucose, suggesting that virulence down-regulation may be coordinated with transition to 'persistence'. We previously demonstrated that glucose-limitation did not modulate transcription of plasmidresponsive loci in *C. muridarum*, so we hypothesized that this species would not regulate *omcAB* transcription in response to environmental stress.

Method
Using *C. muridarum* expressing *omcA::gfp*, we examined the effect of stressors, including 2-deoxyglucose (2DG), penicillin, iron limitation, and hyperosmolality on expression of the fluorescent reporter. 2DG-treated, infected cells were fixed and stained 28 or 40 hours post infection with anti-Pgp3 or anti-OmcB antibodies to assess protein expression. The impact of stress on production of infectious progeny was quantified. The *C. muridarum* reporter fusion was introduced into *C. trachomatis* L2/434/Bu and the assays repeated to determine if dysregulated transcription resulted from a defective *omcA* promoter.

Results
We observed GFP fluorescence under all conditions assayed, indicating that *omcAB* transcription by *C. muridarum* was unaltered in response to stress. Immunofluorescent staining revealed that 2DG-treated *C. muridarum* displayed reduced OmcB expression and altered RB morphology but Pgp3 expression and secretion was detected. Administration of 2DG, penicillin, and iron deficiency resulted in lower levels of infectious progeny. The reporter became stress responsive when assayed in *C. trachomatis*.

Conclusions
These results indicate that post-translational pathways leading to persistence are active in *C. muridarum* but that coordinated down-regulation of virulence-associated gene transcription in response to stress, detected in the human pathogen *C. trachomatis*, does not occur. These findings may contribute to the contrasting disease severity and speed of pathogen clearance observed between humans and mice.

S-08 Coxiella burnetii macrophage infectivity potentiator protein: Evaluation of a novel target for therapeutic intervention

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Background
Coxiella burnetii is a Gram-negative intracellular pathogen that causes Q fever, a debilitating disease in both animals and humans. The only available vaccine, Q-Vax, has limited use as a countermeasure to contain outbreaks due to the risk of severe adverse reactions, thus the identification of new drug targets is essential. Macrophage infectivity potentiator (Mip) proteins catalyze the folding of proline-containing proteins through their peptidyl prolyl *cis-trans* isomerase (PPIase) activity. Mip has been shown to play an important role in the virulence of several pathogenic bacteria but to date has not been investigated as a drug target in *C. burnetii*.

Method
The Mip homologue in *C. burnetii*, *Cb*Mip (CBU0630), was recombinantly expressed and a protease coupled PPIase assay was used to test its activity. A group of pipecolic acid derived compounds designed to inhibit Mip proteins were screened for potency against *Cb*Mip and evaluated against *C. burnetii* strains NMI and NMII in axenic media. The activity of these compounds was also tested against strain NMII in cell infection assays and in the *Galleria mellonella* larvae infection model.

Results
Pipecolic acid derived compounds demonstrate inhibitory properties against *Cb*Mip. These compounds were found to significantly inhibit intracellular replication of *C. burnetii* NMII in both HeLa and THP-1 cells over 5 days as determined by qPCR and intracellular staining of infected cells as compared to control infected cells. Mip inhibitors were also found to inhibit the growth of both NMI and NMII strains in axenic culture. Furthermore, Mip inhibitors demonstrated protective activity *in vivo* and significantly improved the survival of *Galleria mellonella* infected with *C. burnetii* NMII.

Conclusions
Our data shows that inhibition of Mip represents a potentially novel target for antimicrobial therapies against intracellular pathogens such as *C. burnetii*.

M-12 **Molecular pathogenesis of *Ehrlichia ruminantium* Infection**

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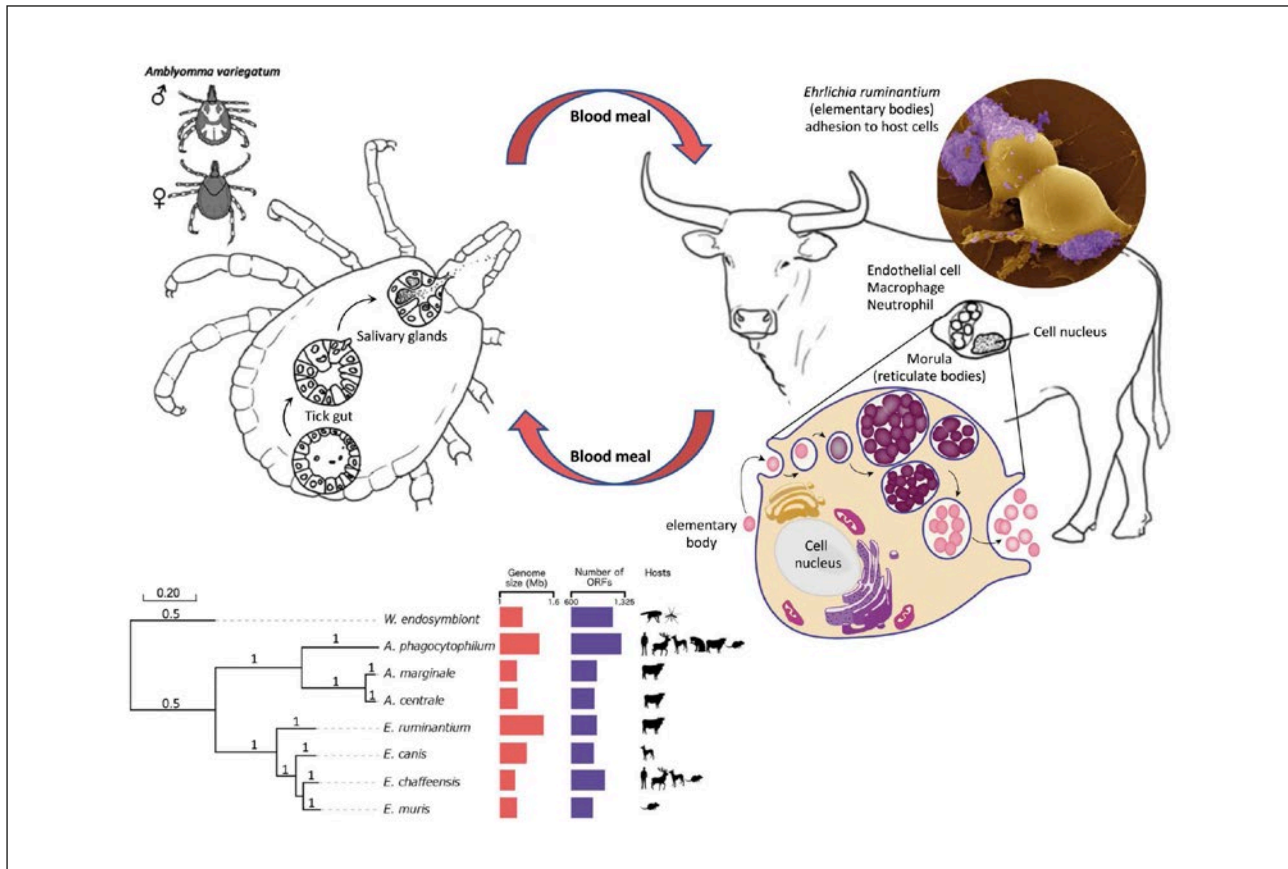
Ehrlichia ruminantium is an obligatory intracellular and tick-borne bacterium that has evolved special proteins and functions to proliferate inside mammalian endothelial cells and cause heartwater disease. To elucidate the molecular mechanisms manipulating host signaling pathways, most notably for immune evasion and nutrients scavenging, we are using a systems biology approach with particular emphasis on the interface between the bacterium and its mammalian or arthropod host.
E. ruminantium induces infectious entry using its entry-triggering protein that bind the mammalian cell surface. During intracellular replication, *E. ruminantium* is able to sense changes in iron concentrations in the environment and to regulate accordingly the expression of virulence factors (type IV secretion system and multigene family of major outer membrane proteins) through a master regulatory gene. A response regulator belonging to a functional pair of two-component system is expressed and regulates the virulence trait of the bacterium. A novel type IV secretion effector of *E. ruminantium* is phosphorylated in the cytoplasm of the host cell and targeted to the nucleus to bind chromatin targets. PNA silencing of this secreted effector blocks *E. ruminantium* infection. Recent results using RNA-sequencing show that inhibition of autophagy also suppresses infection of bovine endothelial cells by *E. ruminantium* and that the bacterium activates cholesterol biosynthetic pathways. The central role of tick saliva in immunomodulation of the host, suggests that *E. ruminantium* have unique infection routes. Comparative analysis of *Ehrlichia* effectomes showed strikingly that genomic plasticity is a major driver to acquire and evolve new potential virulence functions. Further experimental determination of minimal *bona fide* repertoires required for *Ehrlichia* to replicate in a given host is needed.
A major challenge in the future will be using systems-level knowledge in *Ehrlichia* genomics, to predict the potential threat of emergence or to imagine science-based rapid response plans.

Keywords
outer membrane proteins, type IV secretion systems, two-component system, adhesin, effector, iron, cholesterol, autophagy, gene silencing, RNA-seq



M-13 **The value of Culture Collections in The Microbial Sciences: The Importance of ensuring that Intracellular Pathogens are well represented**

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Culture Collections (CC) are Biological Resource Centres (BRC) which facilitate the long-term preservation of biological materials and associated metadata. They have a remit to provide authenticated materials to support transparency, reproducibility, and equity within scientific research. Bacterial CC specifically, have an additional role in that they are fundamental in the advancement of bacterial taxonomy: scientists describing new species must comply with the International Bacterial Code, where it is dictated that submission of a strain (the "Type Strain") to a recognised CC is a prerequisite of defining a bacterial species. Other important functions of CC are to ensure that relevant control strains, alongside recently circulating clinical strains are available to scientists and to provide resources for long term strain archiving. CC also have the specific advantages of having niche expertise in both quality assurance to industry standards (e.g being able to provide an audit trail for strain batch production) and regulatory compliance (eg. international shipping of infectious materials).

Pathogenic obligate intracellular bacteria (eg. Chlamydia spp., Anaplasma spp., *Ehrlichia* spp., etc.) pose a significant threat to both human and animal health. Despite the huge medical and veterinary significance of these agents, they remain significantly underrepresented in global CC, both in terms of strain breadth and depth. This is ultimately due to both a lack of appropriate facilities and expertise within CCs to house bacterial strains which are often complex and expensive to propagate and maintain. Some strains also have the additional regulatory requirements around biosecurity for containment, control, and provision. Addressing this imbalance is fundamental in ensuring that CC remain fit for purpose and can respond to ever changing public health and biosecurity threats.

S-09 Interpretation of the results of Q fever ELISA tests in domestic ruminants: a user-friendly Shiny application based on latent class models in a Bayesian framework

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Background

Despite being recommended for the serological diagnosis of *Coxiella burnetiid* infection in domestic ruminants by the OIE, recent investigations about the diagnostic accuracy of the three ELISA tests currently commercialized showed that these tests are moderately sensitive (ranging between 40% and 94%) and that their respective specificity is inferior to 100% (ranging between 95% and 99%). These results suggest that diagnostic uncertainty should be considered when interpreting the results of these ELISA tests to limit potential false negative or false positive classification of the individual or herd serological status. The objectives of this study were (1) to build a methodological framework allowing calculating predictive values of Q fever ELISA tests at both the individual and the herd levels, and (2) to provide a user-friendly application that could be easily used to interpret the results of a serological sample plan applied to a ruminant herd.

Method

We developed an advanced computing method based on latent class modeling, implemented using JAGS and R to calculate predictive values corresponding to the results obtained with any of the three commercialized ELISA tests, at both the individual and the herd levels. This method was integrated within an open-source web application, using Shiny, to favor its accessibility to all the potential users of these ELISA tests (e.g., veterinarians, veterinary diagnostic laboratories).

Results

After completing the characteristics of the herd and the Q fever epidemiological context (if known), the users obtain the probability of true seropositivity of the tested herd and animals given the ELISA test results.

Conclusions

This application allows a proper interpretation of the results of Q fever ELISA tests according to the sampling size and to available epidemiological and herd information. This application could also be useful for local and reference laboratories for the confirmatory diagnosis of an ELISA test result.

S-10 EVOO-based formulations against Chlamydiae: a promising approach

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Background

Extra virgin olive oil (EVOO), a cornerstone in the Mediterranean diet, is well-known for its nutritional and health properties, and, recently, has been increasingly studied for its anti-bacterial properties. To date, no studies have been performed on its activity against obligate intracellular bacteria, like *Chlamydia trachomatis*, leading cause of bacterial sexually transmitted diseases, and *C. pneumoniae*, responsible for respiratory infections, like pneumonia. In the last decades, clinical treatment failures to antibiotics have been reported, and, hence, novel therapeutic approaches need to be investigated. Therefore, here we evaluated, for the first time, the anti-chlamydial activity of a green EVOO-based extract in natural deep eutectic solvent (NaDES), as well as of its main polyphenol components, namely purified oleocanthal and oleacein in NaDES.

Method

Different concentrations of EVOO extract (170 µg/mL, 17 µg/mL and 1.7 µg/mL), oleacein and oleocanthal in NaDES (55 µg/mL, 5.5 µg/mL and 0.55 µg/mL) were tested against *C. trachomatis* serovar D strain UW3 and *C. pneumoniae* strain IOL-207, in different phases of their growth cycle: *i.* pre-treatment of chlamydial Elementary Bodies (EBs); *ii.* pre-incubation of host cells; *iii.* treatment of chlamydial infected cells.

Results

EVOO extract in NaDES (170 µg/mL) showed the highest anti-chlamydial activity against *C. trachomatis* and *C. pneumoniae* EBs (decrease of the number of IFU/field 93.5% and 91.1%, respectively, $p<0.01$). Oleacein and oleocanthal (55 µg/mL) also showed efficacy ($p<0.05$) during the pre-treatment of chlamydial EBs. No anti-chlamydial activity was observed following pre-incubation or treatment phases.

Conclusions

The direct effect of EVOO extract, as well as of oleacein and oleocanthal, in NaDES, against *C. trachomatis* or *C. pneumoniae* EBs, suggests EVOO as potential preventive strategy for reducing Chlamydiae transmission in the population and number of new chlamydial infections.

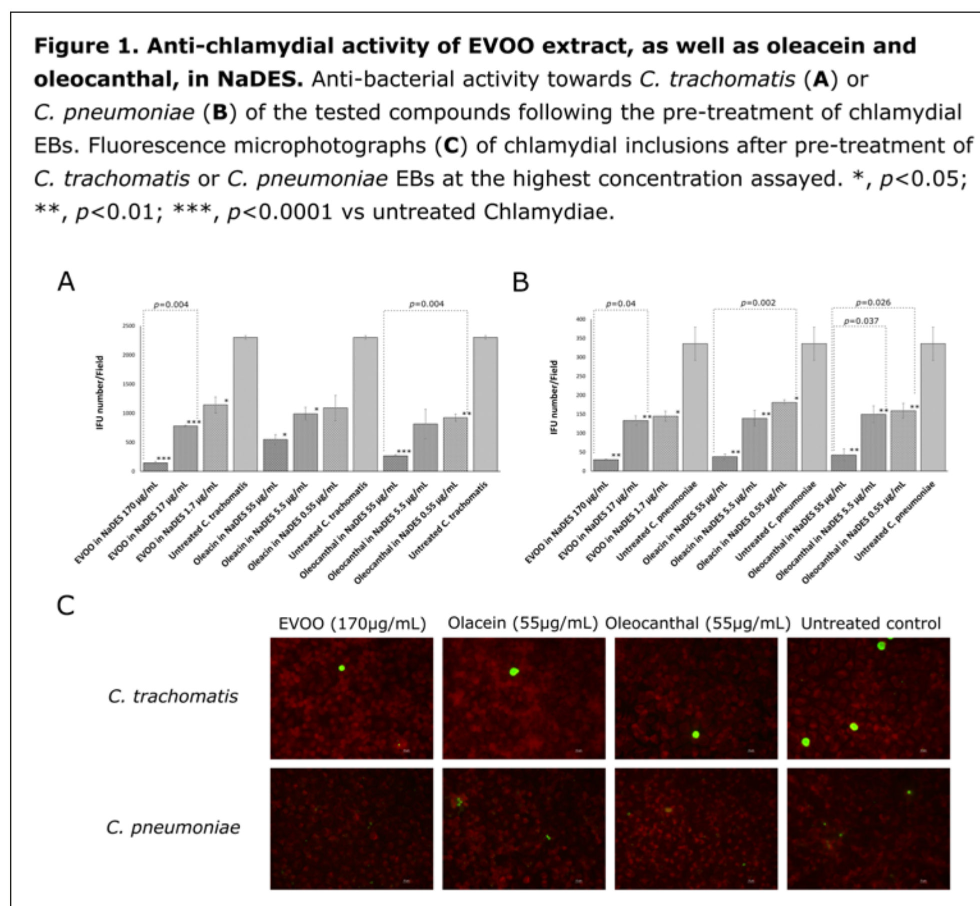

Figure 1. Anti-chlamydial activity of EVOO extract, as well as oleacein and oleocanthal, in NaDES. Anti-bacterial activity towards *C. trachomatis* (**A**) or *C. pneumoniae* (**B**) of the tested compounds following the pre-treatment of chlamydial EBs. Fluorescence microphotographs (**C**) of chlamydial inclusions after pre-treatment of *C. trachomatis* or *C. pneumoniae* EBs at the highest concentration assayed. *, $p<0.05$; **, $p<0.01$; ***, $p<0.0001$ vs untreated Chlamydiae.

M-14 **The chlamydial peptidoglycan remodeling machinery**

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The obligate intracellular *Chlamydiaceae* do not need to resist osmotic challenges inside their host-derived vacuole and thus lost a cell wall in the course of evolution. Instead, they synthesize peptidoglycan (PG) to support an unusual mechanism of cell division. The process of cell division is initiated by a budding mechanism and the asymmetric cell poles mature into two approximately equally sized daughter cells separated by a septum which contains a PG ring. The transient PG ring is remodeled during constriction and degraded afterwards. Recycling of PG appears to be important for *Chlamydiaceae* to maintain a complete cycle of PG-ring synthesis and cell division. Moreover, β-lactams do not kill the genome-reduced pathogens but block cell division and induce reversible 'persistence'. The underlying mechanisms of this phenomenon are not fully understood.
We explored the biological function of the chlamydial PG biosynthesis pathway and linked conservation of PG precursor lipid II cycling to cell division. We showed that cell division amidase AmiA from *Chlamydia pneumoniae* is a novel penicillin target acting both as carboxypeptidase and amidase releasing peptide side chains from the PG sugar moieties. We also identified LysM protein CPn0902 and chlamydial PBP6 as penicillin-sensitive carboxypeptidases. In addition to new findings on chlamydial cell division amidases, a summary on our recent research on the PG-ring remodeling machinery, including novel PG-peptide recycling enzymes and putative PG-binding proteins, will be given.
All in all, chlamydiae may emerge as model systems to understand how a minimal and modified PG machinery supports prokaryotic cell division. Moreover, recycling of PG ring material is likely involved in the recovery of energy-cost intensive intermediates and might control release of immunogenic PG material, contributing to sustaining long-term residence inside the host.

M-15 **Plasma membrane under attack: how *Chlamydiae* enter host cells**

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For obligate intracellular pathogens, adhesion to and internalization of host cells is of utmost importance. Work over the last years has shown that *Chlamydiae* use a complex combination of adhesin – receptor interactions and type-three-secreted effector proteins to ensure successful uptake by non-professional phagocytic cells such as epithelial host cells.
The first attachment of the infectious chlamydial elementary bodies (EB) to target cells is mediated by the conserved adhesin OmcB, which interacts with host heparan sulfate-like proteoglycans. GAG specificity of *C. trachomatis* (*Ctr*) OmcB reflects biovar-specific differences, which might account for tissue tropism and pathogen spread. The *Ctr* adhesin and invasin Ctad1 binds the integrin β1subunit receptor, inducing receptor signaling and activation of ERK1/2. The most prominent chlamydial adhesins are the family of polymorphic membrane proteins (Pmp). The nine Pmp proteins in *Ctr* and 21 Pmp proteins in *C. pneumoniae* (*Cpn*) have been subdivided on phylogenetic grounds into the six subtypes A to H. All 9 *Ctr* serovar E Pmp proteins mediate adhesion to epithelial and endothelial human cells and they are important for infection. In *Cpn*, the adhesin and invasin Pmp21 binds to and activates the epidermal growth factor receptor. Biochemical evidence points to adhesioncompetent homo- and hetero-oligomerization of *Cpn* and *Ctr* Pmp proteins suggesting a possible mechanism of immune evasion.
Recently we identified the *Cpn*-specific adhesin LipP, which acts as a bacterial phosphatidylserine (PS) translocator capable of specific and apoptosis-independent PS externalization during infection and *in vitro* at synthetic membranes. Translocated PS in the outer leaflet of the plasma membrane (PM) may act as a specific host-cell ligand, to which the LipP adhesin on the EB cell surface can then bind. Moreover, translocation of PS will also modulate PM structure underneath the invading EB to support formation of the developing endocytic vesicle.
During entry, *Cpn* also manipulates the PM of the host cell by secreted effector proteins. CPn0677 is secreted within 15 min post infection and binds to the inner leaflet of the invaginating host's PM, induces inwardly directed, negative membrane curvature, and recruits the membrane-deforming BAR-domain containing proteins Pacsin and SNX9. In addition, while bound to the membrane, CPn0677 recruits monomeric G-actin, and its Cterminal region binds and activates N-WASP, which initiates branching actin polymerization via the Arp2/3 complex. Together, these membrane-bound processes enable the developing endocytic vesicle to engulf the infectious EB, while the associated actin network generates the forces required to reshape and detach the nascent vesicle from the PM. Cpn0677 (now renamed SemD) is the first example of a scaffolding effector protein acting centrally on so many components of the endocytic machinery.
Thus, Cpn enters host cells by a unique combination of extracellular adhesins and secreted effectors, which target and manipulate the cell´s PM in a variety of ways.

S-11 Molecular and functional analysis of Bartonella henselae host cell adhesion

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Background

Bacterial adhesion to the host is the most decisive step in infections. Trimeric autotransporter adhesins (TAA) are important pathogenicity factors of Gram-negative bacteria. The prototypic TAA Bartonella adhesin A (BadA) from human pathogenic Bartonella henselae mediates bacterial adherence to endothelial cells (ECs) and extracellular matrix proteins.

Method

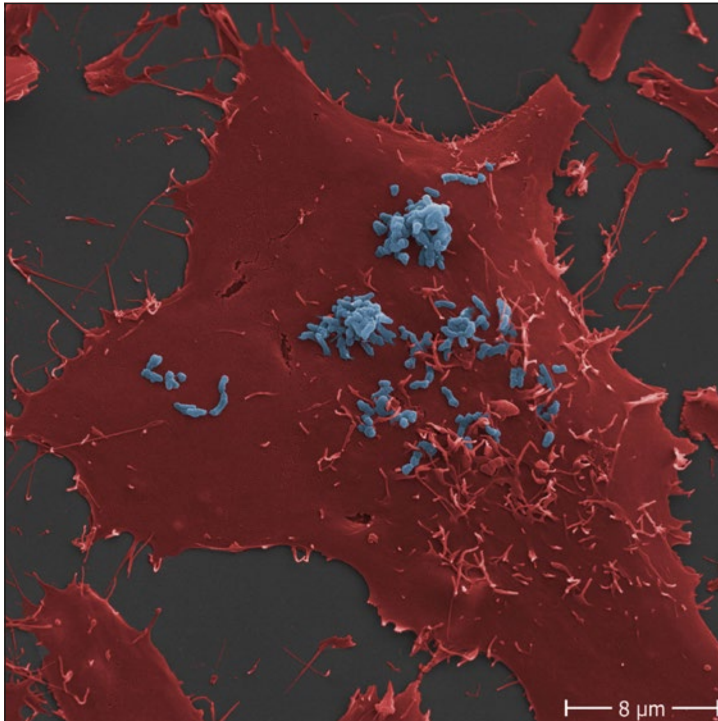
Here, we determined the interaction between BadA and fibronectin (Fn) to be essential for bacterial host cell adhesion. BadA interactions occur within the heparin-binding domains of Fn.

Results

The exact binding sites were revealed by mass spectrometry analysis of chemically crosslinked wholecell bacteria and Fn. Specific BadA interactions with defined Fn regions represent the molecular basis for bacterial adhesion to ECs and these data were confirmed by BadA-deficient bacteria and CRISPR-Cas knockout Fn host cells.

Conclusions

Interactions between TAAs and the extracellular matrix may represent the key step for adherence of human pathogenic Gram-negative bacteria to the host.



S-12* The Orientia tsutsugamushi ScaB autotransporter protein is required for adhesion and invasion of mammalian cells

Nam-Hyuk Cho [1], *Chaewon Kim* [1], Yuri Kim [2], Yen NauyenThihai [2], Jkyeong Jeon [2], Jun-Gu Kang [3], sohee kim [2], Na-Young Ha [4]

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Background
Autotransporter proteins are widely present in Gram-negative bacteria. They play a pivotal role in processes related to bacterial pathogenesis, including adhesion, invasion, colonization, biofilm formation, and cellular toxicity. Bioinformatics analysis revealed that Orientia tsutsugamushi, the causative agent of scrub typhus, encodes six different autotransporter genes (scaA–scaF). Although four of these genes (scaA, scaC, scaD, and scaE) are present in diverse strains, scaB and scaF have been detected in only a limited number of strains. Previous studies have demonstrated that ScaA and ScaC are involved in the adherence of host cells. However, the putative function of other *O. tsutsugamushi* Sca proteins has not been studied yet.

Method
In this study, we show that scaB is transcribed and expressed on the surface of *O. tsutsugamushi* Boryong strain. Using a heterologous Escherichia coli expression system, we demonstrated that ScaB-expressing E. coli can successfully mediate adherence to and invasion into non-phagocytic cells, including endothelial cell and epithelial
cell.

Results
In addition, pretreatment with a recombinant ScaB polypeptide inhibits the entry of *O. tsutsugamushi* into cultured mammalian cells. Finally, we also identified the scaB gene in the Kuroki and TA686 strains and observed high levels of sequence variation in the passenger domains.

Conclusions
We propose that the ScaB protein of O. tsutsugamushi can mediate both adhesion to and invasion into host cells in the absence of other O. tsutsugamushi genes and may play important roles in bacterial pathogenesis.

*Student paper

M-16 **Scrub typhus, update and overview**

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Scrub typhus, a vector-borne febrile illness caused by Orientia tsutsugamushi, is among the leading causes of febrile illnesses in SE-Asia. The causative obligate-intracellular pathogen O. tsutsugamushi has a high genetic diversity, is transmitted by the bite of Trombiculid mites and is increasingly recognized to have a wider global distribution than previously assumed.
In this update and overview the following new findings in scrub typhus research will be presented and discussed; i) a brief update on the burden of disease including new data from SE-Asia; ii) insight on clinical and laboratory predictors as well as ecological risk factors for scrub typhus, and their roles for implementing interventions at the community level - based on recent findings from central Vietnam; iii) the pathogenicity of the O. tsutsugamushi Gilliam strain in an improved Rhesus macaque model providing clinic-pathophysiological disease time course data; and iv) dissecting the diagnostic performance dynamics for qPCR, IgM ELISA and RDTs in a cohort of scrub patients upon hospital admission, highlighting the advantages (and improvement needs) of RDTs as a diagnostic modality at the community health care level in central Vietnam.

M-17 **Typhus and spotted fever: clinical overview**

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Spotted fever group (SFG) and typhus group Rickettsioses are a common cause of unexplained acute fever in endemic settings and of non malaria fever in returned travelers. Humans are infected by the bite of an infected tick, flea, louse or mite. These vector-borne febrile illnesses are mostly re-emerging or newly emerging, with more than 15 pathogenic rickettsioses in SFG; including Rocky Mountain spotted fever, Mediterranean spotted fever, rickettsial pox … In the last 2 decades, TIBOLA/ SENLAT was described and related to other bacteria than Rickettsia slovaca, the most frequent agent. Typhus group includes Rickettsia prowazekii and Rickettsia typhi.
For SFG, the common presentation is an acute febrile illness that associate a characteristic entry wound (eschar) and an erythematous maculo-papular rash, according to the organism and the region.

	Rash %	Rash specificity	Eschar %	Lymph nodes
Rocky Mountain spotted ferver	90	45 % Purpuric	Very rare	No
Mediterranean spotted fever	97	10 % purpuric	72	Rare
Siberian tick typhus	100	Vesicular	77	Yes
Queensland tick typhus	100	8 % purpuric	65	Yes
Israëli spotted fever	100	Vesicular	Rare	No
Flinder's Islands spotted fever	85	-	28	Yes
Astrakhan fever	100	-	23	No
African tick-bite fever	30	-	100, multiple	Yes
Japanese spotted fever	100		90	No
R. mongolotimonae	yes		Yes	No
TIBOLA/ SENLAT	No		Yes	Yes
Murine typhus, R. typhi	60%	macular	No	No
Epidemic typhus, R. prowazekii	100	80% pupuric	No	No

The clinical manifestations of rickettsial disease range from a mild, self-limiting illness to life-threatening multi-organ failure. The fatality rate in untreated cases is 1–15%. It increases with immune suppression, G6PD deficiency, age and the type of Rickettsia involved. The clinical presentation is not sufficiently distinctive for a clinical diagnosis, and clinical scores are useful tool to diagnose and predict severe forms. Definitive diagnosis can be made by serology, IFI the current mainstay of diagnosis, but gives frequently a delayed diagnosis confirmation. An eschar biopsy or swab can be used to demonstrate Rickettsia by PCR.
Antibiotic therapy is highly effective if started early in the disease course. Almost all rickettsial infections respond to doxycycline within 48-72 hours.

S-13* In vitro anti-chlamydial effects of the histamine h1 receptor antagonist azelastine hydrochloride

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Background
We have previously shown that Azelastine hydrochloride (Az), a histamine H1 receptor (H1R) antagonist, exerts anti-chlamydial effects on *Chlamydia trachomatis* (CT) in HeLa cells. In this study, we aimed to gain insight into the mechanism of this interaction and elucidate the role of the H1R in chlamydial development in HeLa cells.

Method
First, we evaluated time-dependent effects of Az by either pre-incubating HeLa cells with Az before infection or by adding Az at 0 - 24 hours post-infection (hpi). Cultures were incubated for 43 hpi, followed by sample collection for inclusion size, number and infectivity analyses. Additionally, we considered host cell specificity of Az anti-chlamydial effects by evaluating Az in an ocular model of chlamydial infection (human conjunctival epithelial cells and CT serovar B). Ultimately, we assessed the role of the H1R in chlamydial development by receptor modulation using a specific H1R antagonist or an H1R agonist for a total of 43 h, followed by chlamydial infection analyses.

Results
While we only observed mild anti-chlamydial effects in the pre-incubation setting, addition of Az up to 12 hpi strongly reduced chlamydial infectivity and changed chlamydial morphology. Interestingly, these effects were more pronounced when Az was added early after infection compared to later time points. Az effects were reproduced in the ocular model. Modulation of the H1R with other pharmaceutics, however, failed to induce similar anti-chlamydial effects.

Conclusions
We conclude that Az effects are independent of the chlamydial strain or host cell. However, our hypothesis that modulation of the H1R limits chlamydial development was not supported, as we neither observed these effects in the pre-incubation setting, nor when modulating the H1R with other H1R-specific pharmaceutics. We therefore conclude that likely off-target effects of Az are relevant for its impact on chlamydial development.

*Student paper

S-14 Purified compounds isolated from a South American tree, Lithraea molleoides (Vell.) Engl., inhibit Chlamydia trachomatis infection in vitro

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Background

Chlamydial infections are widely distributed and are responsible for a variety of acute and chronic diseases, which sometimes lead to complications and sequelae. Currently recommended antibiotic treatment has shown some treatment failures and proven unsatisfactory efficacy in chronic infections. Hence, there is a continuous search for new antichlamydial agents. Recently, we reported the anti-chlamydial activity of an insoluble fraction from the methanol extract of *L. molleoides*, a South American vegetal specie with recognized ethnomedical uses. We aimed to chemically characterize the active fraction of *L. molleoides* and assess its purified compounds' in vitro antichlamydial activity against C. trachomatis.

Method

The insoluble fraction of a methanolic extract of L. molleoides was studied by HPLC-UV DAD, and the compounds detected were identified by IR, MS, 1H, and 13C-NMR spectra, and homo- and heteronuclear correlation spectroscopy. The fraction (100 µg/mL) and their major compounds were assayed: ellagic acid 33,1 µM (100 µg/mL), rutin 163µM (100 µg/mL) and ellagic acid:rutin 1:1 and 9:1. The antichlamydial activity was assessed on cell culture as described by Entrocassi et al. (doi: 10.1016/j.heliyon.2021.e06947) using LLC-MK2 cell line and C. trachomatis ATCC strain L2/434/BU.

Results

Ellagic acid and rutin were identified (90% / 10%, respectively) in the L. molleoides insoluble fraction (Fig 1). Ellagic acid showed the highest inhibitory activity (99%) during the inoculation step of the Chlamydial life cycle (Fig 2). While rutin showed no inhibitory activity, the synergy of both compounds 1:1 improved the effect of the Ellagic acid over the cell culture.

Conclusions

The purified isolated compounds of the L. molleoides fraction showed a high capability to prevent infection by Chlamydia trachomatis in cell culture. While further research is needed on the pathways involved in the ellagic acid impairing upon chlamydia entry, a promising new antichlamydial agent can be developed.

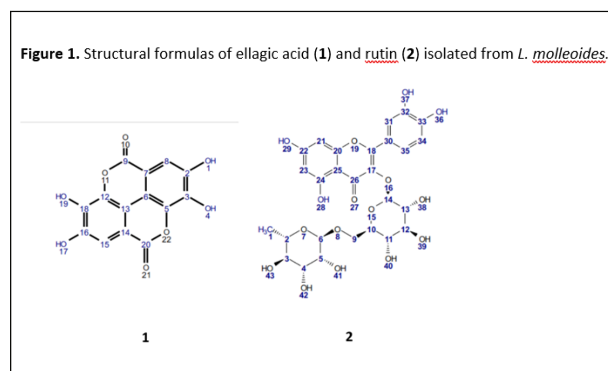

Figure 1. Structural formulas of ellagic acid (**1**) and rutin (**2**) isolated from *L. molleoides*.

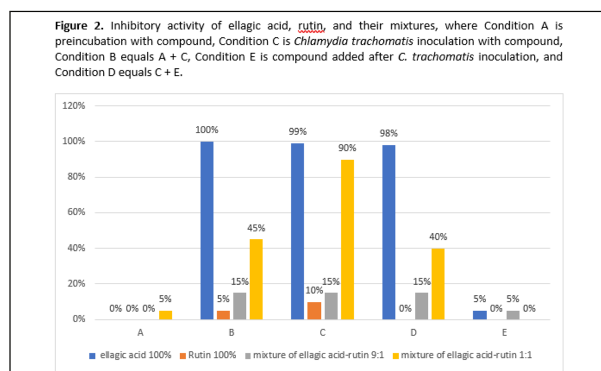

Figure 2. Inhibitory activity of ellagic acid, rutin, and their mixtures, where Condition A is preincubation with compound, Condition C is *Chlamydia trachomatis* inoculation with compound, Condition B equals A + C, Condition E is compound added after *C. trachomatis* inoculation, and Condition D equals C + E.

M-18 The roll of peptidoglycan in division, differentiation, and 'persistence' in *Chlamydia trachomatis*

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Cell size regulation in bacteria is a function of two basic cellular processes: the expansion of the cell envelope and its constriction at spatially defined points at what will eventually become the division plane. In most bacterial species, both cell wall expansion and restriction are dependent on peptidoglycan (PG), a structural polymer comprised of sugars and amino acids that imparts strength and rigidity to bacterial membranes. Pathogenic *Chlamydia* species are unique in that their cell walls contain very little PG, which is restricted almost entirely to the apparent division plane of the microbe's replicative form. Very little is known about the degree to which PG affects the size and shape of *C. trachomatis* and the degree to which changes in the host cell environment affect PG biosynthesis and assembly during its development. Upon conducting an analysis of the dimensions, orientation, and relative density of chlamydial PG throughout the organism's developmental cycle, we found that PG in replicating *C. trachomatis* is dynamic, oscillating between polar-defined disks, enlarging rings, and contracting septa. As *C. trachomatis* progresses through its developmental cycle PG structures decrease in total volume and have lower rates PG biosynthesis to turnover, indicating that the average cell volume of chlamydial RBs likely decreases over time. The targeted inhibition of either of the microbe's two PG synthases results in drastic changes in the ratio of PG synthesis to degradation, as well as the average volume and shape of PG-containing structures. These results suggest that *C. trachomatis* PG synthases differentially regulate the expansion and contraction of the PG ring during both the expansion and constriction of the microbe's cell membrane during cell growth and division, respectively. *C. trachomatis* also utilizes a variety of mechanisms to dampen the immunological recognition of its PG, and we have determined that this immunoevasive feature is particularly apparent during conditions collectively used to describe the organism's 'persistent' state.

M-19 **Investigation of chlamydial division mechanisms using Chlamydia-related bacteria as a model**

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Bacterial division is usually organized by FtsZ, a homologue of the eukaryotic cytoskeleton component tubulin. FtsZ forms a ring at the division septum and further recruits components of the divisome machinery, which coordinates peptidoglycan remodelling and membrane invagination. Interestingly, some bacterial species, including Chlamydiae, lack a FtsZ homologue.

Chlamydiae are obligate intracellular bacteria with a peculiar biphasic developmental cycle. The *Chlamydiales* order is composed of well-characterized pathogens such as *Chlamydia trachomatis* and *Chlamydia pneumoniae*, members of the *Chlamydiaceae* family. Other members of the *Chlamydiales* order are called Chlamydia-related bacteria. They can have very diverse hosts, from amoebae to mammals, and some of them are considered as emerging pathogens. *Waddlia chondrophila* is a *Chlamydia*-related bacterium, which was first isolated from aborted bovine fetuses. *W. chondrophila* can grow in a wide variety of host cells, from amoebae to human cell lines. Moreover, it has a larger cell size than *Chlamydiaceae*, facilitating the localization of divisome components. Finally, this bacterium is, in contrast to *Chlamydiaceae*, sensitive to phosphomycin, an antibiotic inhibiting the first step in the synthesis of peptidoglycan. Taken together, this makes *W. chondrophila* an interesting model to investigate chlamydial division.

Using this model, we identified several members of the chlamydial divisome and could determine their localization and activity. Our results also brought a better understanding of the importance of peptidoglycan for the organization of the chlamydial divisome and allowed the identification of peptidoglycan-modifying proteins, which are important for chlamydial division.

Despite recent advances, much still needs to be investigated, especially regarding the regulation of chlamydial division and what signals regulate division during the chlamydial developmental cycle.

S-15* Anaplasma phagocytophilum exploitation of host-cell multivesicular body pathways facilitates bacterial replication and dissemination

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Background

Anaplasma phagocytophilum is a vacuole-adapted obligate intracellular bacterium that causes the emerging disease, human granulocytic anaplasmosis. The *A. phagocytophilum* vacuole (ApV) interfaces with membrane trafficking pathways between numerous host cell organelles in a manner that parallels the multivesicular body (MVB). Accordingly, we investigated the involvement of the endosomal sorting complex required for transport (ESCRT) machinery in the biogenesis of MVBs and specific Rab GTPases required for trafficking MVBs in the context of *A. phagocytophilum* infection.

Method

Transmission electron and immunofluorescence microscopy were used to evaluate protein localization to the ApV. The effects of siRNA silencing of ESCRTs on bacterial load, ApV maturation, infection cycle progression, and infectious progeny release were assessed using qPCR, Western blot, and immunofluorescence. The ability of Nexinhib20, which blocks Rab27a-mediated MVB exocytosis, to impair *A. phagocytophilum* release was also examined.

Results

Analogous to an MVB, the ApV is an acidic compartment that contains intraluminal vesicles, lysobisphosphatidic acid, and is decorated with ESCRTs. Knocking down expression of critical subunits in the ESCRT-dependent or -independent MVB biogenesis pathway failed to negatively impact infection. However, simultaneously targeting both pathways significantly hindered *A. phagocytophilum* replication and infectious progeny generation. Knockdown of CHMP4, a subunit where the two pathways converge, drastically reduced sorting of host vesicular membrane markers into the ApV and halted bacterial growth, infection cycle progression, and infectious progeny production. Knockdown of Rab27a, a GTPase essential for exocytosis from MVBs, did not affect bacterial replication but significantly reduced infectious progeny exit, as did Nexinhib20.

Conclusions

Our data define the ApV as a modified MVB that exploits ESCRT machinery to deliver host cell vesicular traffic into the organelle, which fuels *A. phagocytophilum* intracellular replication and infectious progeny generation. Additionally, the pathogen exploits Rab27a-dependent exocytosis from MVBs for dissemination and hence further propagation of infection.

*Student paper

S-16* New insights into chlamydial cell division amidase function, architecture and inhibition

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Background
Chlamydia trachomatis is the most widespread sexually transmitted bacterial pathogen and the leading cause of preventable blindness worldwide. Due to its obligate intracellular lifestyle, *C. trachomatis* does not have to withstand environmental challenges like free-living bacteria, and therefore lacks a cell wall. Instead, the genome-reduced organism synthesizes a transient peptidoglycan ring for cell division, which is degraded afterwards. Peptidoglycan-ring constriction, degradation and recycling requires constant remodeling including multiple enzymatic steps.

Method
Please see below as integrated in the results section.

Results
Here, we show that AmiA, the only cell division amidase retained in *Chlamydia*, is involved in the peptidoglycan remodeling process in *C. trachomatis*. Surrogate host experiments in *Escherichia coli* revealed that heterologously overproduced AmiA from *C. trachomatis* (AmiA_Ct) lyzes the *E. coli* producer strain. Expression of *amiA*_Ct in an *E. coli ΔamiABC* triple knockout mutant rescued cell separation and averted a chainforming phenotype, presumably by degradating septal PG during cell division. Enzymatic *in vitro* experiments with the purified protein confirmed a hydrolytic activity on peptidoglycan. In contrast to bifunctional AmiA from *C. pneumoniae*, AmiA_Ct showed monofunctional amidase activity on the peptidoglycan precursor lipid II. Using site-directed mutagenesis, we analyzed differences in the zinc-coordinating active site of AmiA_Ct as compared with *C. pneumoniae* AmiA. Pulldown experiments with wild type and active site mutant proteins indicated that peptidoglycan binding is independent from enzymatic functioning in AmiA_Ct. Moreover, we identified potent inhibitors of AmiA_Ct, including the antibiotic clioquinol. These compounds were used as tools to study functioning of AmiA_Ct in cell culture infection models.

Conclusions
Our work contributes to the understanding of chlamydial amidases including the role they play in the tightly orchestrated peptidoglycan biosynthesis and cell division machinery.

*Student paper

M-20 **The epidemiology and treatment of scrub typhus and murine typhus**

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Scrub typhus (caused by *Orientia tsutsugamushi*) and murine typhus (caused by *Rickettsia typhi*) are important but neglected causes of fever, both in patient management and in research. Recent research on their epidemiology will be reviewed, gaps in the evidence base examined and the impact of climate change on their spatial and temporal distribution discussed. Evidence from the Lao PDR suggesting that climate change will have important impact on both diseases will be described. Prospective investigation is needed of risks and mitigation strategies. The importance of including both diseases in routine surveillance will be discussed and, building on the recent discovery of scrub typhus in Chile, the risks that this may be unrecognized outside of the known endemicity.

Recent data on the optimal treatment of both diseases will be discussed and their implications for both targeted treatment and empirical therapy of fever in endemic areas reviewed.

S-17 Variability of chlamydia trachomatis genotypes from the argentinean lymphogranuloma venereum outbreak detected in Buenos Aires in 2017-2019

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Background
Lymphogranuloma venereum (LGV) is a systemic sexually transmitted disease caused by L1 to L3 *Chlamydia trachomatis* serotypes. Since 2003 an increase in LGV cases in European countries was observed, where the predominant genotype was L2b. First cases were detected in Buenos Aires, Argentina in 2017. Our goal was to characterize *C. trachomatis* genotypes detected in anorectal infections from patients with proctitis-related symptoms in Buenos Aires.

Method
Between 09/2017 and 08/2019, 239 samples from symptomatic patients were included in this study. Anorectal swabs were tested for *C. trachomatis* by real-time PCR targeting the cryptic plasmid. Positive samples were typed by *omp*A PCR-RFLP and further *omp*A sequencing.

Results
Chlamydia trachomatis infection was detected in 44.77% (107/239) of the anorectal swabs being 104/221 MSM, 2/10 trans-women, and 1/8 women. All patients reported having had unprotected sexual intercourse and almost all were HIV+ under treatment. *omp*A sequencing was successful in almost all positive samples (104/107). *omp*A sequencing showed that 84.62% of the infections were caused by LGV genotypes and 15,38% by non-LGV genotypes. Almost one-half of the LGV positive samples were genotype L2b, a third were genotype L1 -only sporadically detected in the European outbreak-, and a quarter were genotype L2 (table 1). Among the 38 L2b samples, 29 had an identical *omp*A genotype, and 9 showed 1or 2 SNPs difference not previously described (4 were identical, and the other five had different SNPs). Of the 27 L1 genotype samples, 26 were identical, and one had an SNP difference. Finally, L2 samples were identical, except one containing an SNP variation (table 2).

Conclusions
The LGV outbreak detected in Buenos Aires has a unique genotype distribution. L2b genotypes showed high sequence variability, and an exceptionally high L1 genotype proportion was detected.

Table I. Distribution of *Chlamydia trachomatis omp*A genotypes in anorectal infections in patients with symptoms consistent with proctitis in Buenos Aires, Argentina (2017-2019).

		SAMPLES (N)	PERCENTAGE
Total		239	
Real time PCR Positives		107	44.77%
***omp*A PCR – RFLP**			
LGV genotipes		88/107	82.24%
Non-LGV genotipes		19/107	17.76%
***omp*A SEQUENCING**			
Analyzable Sequences		104/107	97.20%
GENOTIPES			
Non-LGV *		16/104	15.38%
LGV	Total	88/104	84.62%
	L2b	38/88	43.18%
	L1	27/88	30.68%
	L2	23/88	26.14%

* Non-LGV genotypes detected include D (n=5), G(n=3), J (n=3), F (n=2), E (n=1), H (n=1), and one mix infection that includes the Ja genotype in higher proportion.

Table II. Variability of the *omp*A gene in *Chlamydia trachomatis* LGV2b detected in anorectal infections from patients with symptoms compatible with proctitis in Buenos Aires, Argentina (2017-2019).

SAMPLES (N)	SNP*	MOLECULAR CHARACTERIZATION
29	0	100% identical to the sequence JN795427.1*
4	1	JN795427.1:c.517C>A
1	1	JN795427.1:c.490A>G
1	1	JN795427.1:c.802G>A
1	1	JN795427.1:c.1000G>A
1	2	JN795427.1:c.271G>A/JN795427.1:c.493C>A
1	2	JN795427.1:c.494A>G/JN795427.1:c.515A>C

* L2b reference GenBank sequence JN795427.1: *Chlamydia trachomatis* serovar L2b major outer membrane protein gene, partial cds.

GenBank accession numbers MN548736.1 to MN548759.1, MN537150.1 to MN537152.1, and MN563608.1 to MN563615.1

S-18 Genotyping of Orientia tsutsugamushi using complete TSA56 gene: A report from South India

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Background

The immunodominant 56 kDa gene of *Orientia tsutsugamushi,* is useful for genotyping as it has four variable regions (VD-I to VD-IV). In India, genotyping has been based on phylogenetic analysis of 56 kDa partial gene segments. Obtaining complete 56 kDa gene sequence information is important for knowing the circulating strains and for designing region specific diagnostics and vaccines. This study to analyse the complete 56 kDa gene was undertaken to determine *Orientia tsutsugamushi* genotypes circulating in and around Vellore.

Method

Of the 324 buffy coat samples from suspected scrub typhus patients, 140 were positive by 47 kDa qPCR. Samples with Ct value <30 were subjected used to amplify the complete 56 kDa gene using two sets of primers which amplified two overlapping fragments of this gene. The samples which showed clear bands (1000-1100bp) for both the fragments (first half and second half) were Sanger sequenced. Phylogenetic analysis was performed using appropriate reference sequences to determine the genotype.

Results

Among 73 samples with Ct <30, in 27 samples the two TSA 56 gene segments were amplified. Of the six samples sequenced, BLAST result showed 95 to 99% homology with 56 kDa complete gene sequence of *Orientia tsutsugamushi*. The GenBank accession numbers are OK545864, OK642783-86 and OL631134. Of these 5 sequences belonged to TA763 genotype while one belonged to Karp genotype. In two of the TA763 genotypes complete gene sequence (1605 bp) was obtained, while the remaining four had almost complete sequences (1551-1596 bp).

Conclusions

Among the six 56 kDa gene amplicons analysed, in two complete 56 kDa sequences were obtained, both belonging to TA763 genotype. The lone Karp genotype was 1587 bp in size. This is the first data from South India with complete and near complete 56 kDa gene data of *Orientia tsutsugamushi*.

Table 1. Details of the six sequenced 56 kDa amplicons

Sequence ID	Sequence length (ORF) bp	Amino acid	Genotype	Genbank accession number
OT1	1832 (1590)	530	TA763	OK545864
OT2	1830 (1587)	529	Karp	OK642783
OT3	1551 (1551)	517	TA763	OK642784
OT4	1773 (1605)	535	TA763	OK642785
OT5	1795 (1596)	532	TA763	OK642786
OT6	1773 (1605)	535	TA763	OL631134

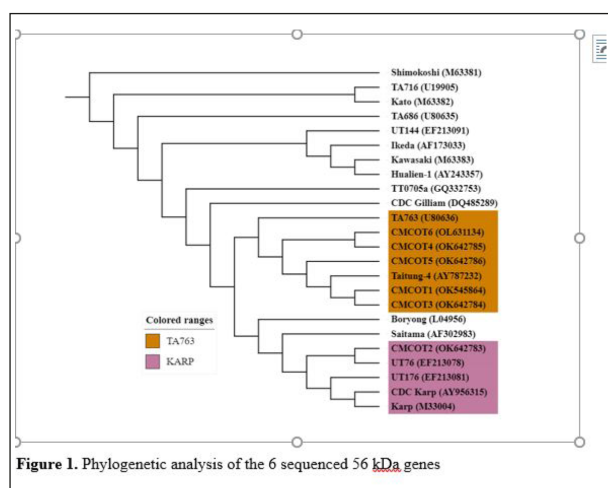

Figure 1. Phylogenetic analysis of the 6 sequenced 56 kDa genes

S-19 Borreliosis in Algeria: Update and comprehensive overview

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Background
The genus *Borrelia* currently contains more than 30 genospecies, which are transmitted by arthropod vectors and exhibit different degrees of pathogenicity. This genus was traditionally classified into two groups: Lyme borreliosis (LB), *Borrelia burgdorferi* sensu lato complex, transmitted principally by ticks of the genus *Ixodes*, and relapsing fever *Borrelia* (RFB), transmitted primarily by argasid ticks and also by lice. In Algeria, LB, RFB and other bacterial pathogens transmitted by ticks are poorly known and epidemiologic data are scarce according to the immense geographical extent of the biggest country in Africa.

Method
The information gatheredwas obtained via online literature searches using the PubMed and Google search engines. The majority of the results represent our personal or team's research work

Results
In human, the first human case of RVB has been reported by Sergent in 1908. Recently, Blood samples collected from febrile patients in Oran, revealed the presence of a new *Borrelia* sp. named Candidatus *Borrelia algerica.* In animals, sheep and goat blood from the north-eastern Algeria were positive from *Borrelia theileri*. Another study in the same region indicated that blood samples of domestic animals were positive for *Borrelia* sp. Actually, only these two reports from the same region indicated the evidence of Borreliae in animals. In argasid ticks, *Borrelia turicatae* has been detected in *Ornithodoros capensis* s.l. in Algiers. The avian spirochete *Borrelia anserin* has been detected in *Argas persicus* from Medea region. Also, the relapsing fever bacterial agents: *Borrelia hispanica* and *Borrelia crocidurae* have been detected in *Ornithodoros occidentalis* and in *Ornithodoros sonrai* in Mostaganem and estern Algeria respectively. Only one of the Lyme disease agent *Borrelia garinii* has been detected in *Ixodes ricinus* from the north-eastern part of the coutry.

Conclusions
Finally, understanding Borreliosis will certainly contribute to prevent the reoccurrence and spread of the infection among humans and animals in Algeria.

S-20 Chlamydia gallinacea as emerging pathogen in poultry

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Background

Chlamydiaceae are Gram-negative bacteria distributed worldwide in animals and humans. Available data show that *C. gallinacea* is dominant chlamydial agent found in poultry in European and Asian countries. Data about *C. gallinacea* are limited, therefore the aim of the studies was screening of poultry flocks in order to evaluate frequency of *C. gallinacea* shedding and genetic diversity.

Method

A monitoring survey of *Chlamydia* spp. dissemination has been performed in poultry in Poland. Sampling was conducted in different regions of Poland in 2019-2020. Overall, 1466 cloacal/oral swabs were collected in duplicate from 146 apparently healthy poultry flocks including chickens, turkeys, ducks, geese and quails. Dry swabs were used for DNA extraction. All DNA extracts were screened using a *Chlamydiaceae* 23S rRNA realtime PCR assay. To identify *Chlamydia* species, specific real-time PCR assays were performed including, e.g. *C. gallinacea* and *C. psittaci*. Furthermore, selected samples were used for sequencing based on *omp*A gene fragment and variable domains (VD1-2, VD3-4).

Results

In total, 10.3% of the tested flocks were *Chlamydiaceae*-positive (15/146 farms). The presence of *Chlamydiaceae* was molecularly confirmed mainly in chickens (13/92 farms). Only one turkey (1/19 farms) and goose (1/26 farms) flock was *Chlamydiaceae*-positive. Eleven flocks were identified as *C. gallinacea*-positive while four flocks remained unclassified. Phylogenetic analysis revealed at least 16 genetic variants of *C. gallinacea*.

Conclusions

Research showed that *Chlamydiaceae* occur in poultry flock in Poland. The strains of *C. gallinacea* as dominant species shows genetic variability.

M-21 ***Chlamydial plasmids***

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A plasmid was first described in Chlamydia trachomatis in the 1980s. It soon became apparent that nearly all isolates of C. trachomatis carried a plasmid. The first plasmid sequences revealed a highly conserved structure suggesting biological constraints on evolution and that the plasmids shared a common ancestor. The ds, circular DNA plasmid is approximately 7,500 nucleotides, encodes eight genes (coding sequences) and exists in a highly supercoiled form, at 4-10 copies per bacterial cell. Rare plasmid-free natural isolates showed that the presence of the plasmid conferred a glycogen + phenotype for Chlamydia trachomatis inclusions. Staining inclusions with iodine to reveal this property was an early method for differentiating C.trachomatis from other species. Later genetic studies showed that genes on the plasmid regulated chromosomally encoded glycogen biosynthesis. In addition, plasmid-bearing strains showed an increased virulence. Thus, and somewhat paradoxically (since the addition of the plasmid and its replication requirements adds a metabolic burden) the presence of the plasmid increases the fitness of the host.

Naturally occurring variants of the Chlamydia trachomatis plasmid are rare and yet in 2006 a new variant emerged in Sweden that had deleted the target sequences of most of the current PCR diagnostics. It was a very clear example of evolution 'by detection and treatment' and reminded us that even in a highly conserved sequence, given the right selection, rapid change was possible. The power of genetics was brought to bear to study plasmid function with the development of a transformation system for Chlamydia trachomatis, initially based on the Swedish plasmid as the backbone for the vector. This allowed significant advances in understanding the biological properties of the plasmid coding sequences since it became possible to systematically delete / engineer sequences to investigate function. Subsequently plasmid based genetic systems have been developed for a range of chlamydial species.

M-22 **Toxin-antitoxin modules in *Waddlia chondrophila* and *Chlamydia*-related bacteria**

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Obligate intracellular bacteria belonging to the *Chlamydiales* order share a biphasic developmental cycle characterised by infectious, non-replicative Elementary Bodies (EBs) and non-infectious, replicative Reticulate Bodies (RBs). Upon exposure to different stressful conditions such as antibiotics, iron deprivation and interferon-g, RBs differentiate into enlarged bacteria called Aberrant Bodies (ABs), which are thought to be involved in chronic infections. Using an RNA-sequencing approach, we determined the transcriptional changes induced by iron starvation in the *Chlamydia*-related species *Waddlia chondrophila*, a potential agent of abortion in ruminants and miscarriage in humans. Among the most up-regulated genes upon iron starvation we identified three type II toxin-antitoxin (TA) modules, two encoded on the *W. chondrophila* cryptic plasmid (a MazEF module and a HigBA module) and one on the chromosome (a HicAB module).
Analysis of the gene expression during the regular developmental cycle and in aberrant bodies induced by different stressful conditions shows that RNA for plasmid-encoded HigBAWc and MazEFWc accumulates in EBs and in ABs induced by iron starvation or novobiocin. Heterologous expression of HigBWc and MazFWc is detrimental to *E. coli* cells, unless the putative antitoxin, encoded by the neighbouring gene, is present. Site-directed mutagenesis identified residues essential for the enzymatic activity of the toxins.
TA systems are thought to play a role in plasmid maintenance and adaptation to different stressful conditions in free-living microorganisms, but are rare in obligate intracellular bacteria, which possess a reduced genome adapted to their specific niche. Strikingly, we could identify putative TA modules on the plasmids of several *Chlamydia*-related species, which suggests that, in spite of the evolutionary pressure towards gene loss, TAs modules are preserved in some strict intracellular bacteria and are likely key players in the response to adverse conditions.

S-21 Development of mutagenesis in diverse species in the genus *Chlamydia*

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Background

Different chlamydial species share many aspects of the typical intracellular life style, such as the biphasic developmental cycle and the preference to primarily invade epithelial cells. However, each chlamydial strain also employs sophisticated species-specific strategies that significantly contribute to an extraordinary diversity in organ and/or tissue tropism and disease manifestation. In order to discover and understand underlying mechanisms of how these pathogens infect particular hosts, utilize nutrients, respond to the eukaryotic intracellular environment, communicate with the host cell, and most importantly, cause specific diseases, it is imperative to utilize a genetic manipulation approach applicable to every chlamydial species.

Method

Generation of deletion mutants by allelic exchange via homologous recombination was achieved by employing a modified pUC19 shuttle vector. pUC19-pL2*ori-Δpmp* or pUC19-pCM*ori-Δpmp,* was generated by linearization of the plasmid with SalI restriction enzyme and ~2.5 kb 5' arm sequences upstream of the target gene, amplified from *C. trachomatis* or *C. muridarum* genomic DNA, were introduced into the vector by Gibson Assembly. Then, ~ 2.5 3' arm sequences, downstream of the gene, were cloned into the plasmid at the SbfI site as described above. Chlamydial transformation and isogenic purification of mutant chlamydiae was performed as previously described.

Results

We generated deletion mutants in *pmpI* and/or *pmpD* (polymorphic membrane protein) genes in various chlamydial strains and species as a proof of principle that our unique strategy of mutagenesis can be broadly employed with essentially any member of the genus *Chlamydia*.

Conclusions

We have developed an adaptable mutagenesis approach broadly applicable to essentially any member of the genus *Chlamydia*. We chose to generate deletion mutants in *pmpI* and/or *pmpD* genes in various chlamydial strains. Chlamydial pmps are immunogenic, autotransporter-like adhesins present in all chlamydiae and thus, are very likely to be involved in initiating infection, disease progression and immune evasion in a species- and/or serovariant-specific manner.

S-22* Cross-feeding interactions shaped Rickettsiales genomic architectures along the parasite/free-living spectrum

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Background
Rickettsiales division harbors microbial lineages accountable for some of the deadliest (and oldest) pestilential diseases of mankind. The early dawn-of-genomics realization that their intracellular lifestyles moulded genomic architectures crystalized later into the mainstream reductive genome evolution paradigm. Though intracellularity, genome shrinking, and impaired metabolic capacity are key conceptual features, the ecological pressures and evolutionary trajectories that generated them remain obscure.

Method
Here, we couple a genome-resolved metagenomic approach (that generated more than 5 500 freshwater prokaryotic genomes) with genome-wide metabolic reconstructions to elucidate the evolutionary history of Rickettsiales and unveil the eco-evolutionary processes that transformed free-living pelagic bacteria into intracellular parasites/symbionts.

Results
Baseline ecosystem-scale taxonomic profiling (based on 214 metagenomic data sets) indicated the existence of novel family-level Rickettsiales lineages with high diversity and abundance in aquatic environments, thus portraying these habitats as diversification hubs of the extant radiation. Deep evolutionary history reconstructions (focused on 50 newly recovered genomes) depicted a pattern of ancestry, divergence, and descent in which iconic intracellular species appear as the latest diversification of a more ancient and larger radiation. Genomeinferred metabolic reconstructions depicted a gradual loss of sensorial and biosynthetic potential along the evolutionary timeline. CARD-FISH imaging associated the major events in phylogenetic diversification and metabolic circuitry degradation with lifestyle transitions from free-living to epi- and endobiotic host associations. Basal free-living clades were found to be enriched in mitochondrial-specific proteins and encompass the closest evolutionary lineages to the pre-mitochondrial ancestor. Thus, showcasing cross-feeding interactions as a reiterating main evolutionary driver in the pre- and post-emergence of Rickettsiales bacteria.

Conclusions
Metagenomic-based approaches are powerful tools to bypass the cultivation-based bottleneck and provide the means to reconstruct the sequences of evolutionary events that pushed free-living environmental microbes towards pathogenesis.

*Student paper

M-23 Development of a new subcellular vaccine to protect sheep from ovine enzootic abortion

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Background
Chlamydia abortus, the aetiological agent of ovine enzootic abortion, is a major cause of reproductive loss in small ruminants. Infection can be controlled through the use of inactivated or live attenuated vaccines, although each has limitations, with the latter associated with disease. We have evaluated the protective efficacy of two experimental subcellular vaccines as safer alternatives.

Method
Four experimental trials were conducted in pregnant sheep comparing the efficacy of two subcellular vaccines (COMC and octyl-glucoside-COMC) to the commercial live vaccine and optimising the most efficacious preparation in terms of number of inoculations, dose and adjuvant. Doses varied from 20 to 2.5 µg antigen. Antigens were formulated in either of two water-in-oil adjuvants (Montanide ISA70VG or ISA61VG) or in QuilA. Sheep were mated and challenged on day 70 of gestation. Cellular (interferon-gamma) and humoral immune responses (to an outer membrane protein) were measured by ELISA. Placentas and foetal tissues were analysed for the presence of the pathogen and associated pathology, while pathogen shedding following delivery was measured by qPCR. Efficacy was assessed as ability of the vaccines to prevent abortion and by a reduction in shedding.

Results
The COMC was determined to be the most efficacious of the two experimental vaccines. The trials demonstrated that the COMC vaccine could be delivered in a single dose equivalent to 10 µg antigen without compromising efficacy and producing improved results in terms of lower shedding and placental pathology compared to the OG-COMC or commercial vaccines. Although, all three adjuvants induced COMC-specific cellular responses, these responses as well as protective efficacy were the greatest with the two Montanide adjuvants.

Conclusion
We have developed and evaluated a new subcellular vaccine based on the COMC antigen that is a safer efficacious alternative to the commercial vaccines. The vaccine is currently being further optimised for commercial-scale production.

M-24 Emerging *Chlamydia* infections in animals and their zoonotic potential

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Chlamydia psittaci and *Chlamydia abortus* are well recognized for their zoonotic potential. *C. psittaci* is an obligate intracellular bacterium that causes respiratory disease in birds. In humans, this organism may cause psittacosis, a respiratory disease that can spread to involve multiple organs, and in rare untreated cases may be fatal. *Chlamydia abortus* is mainly responsible for ovine enzootic abortion, but it is also a dangerous pathogen for pregnant women. A few years ago, *C. abortus* was associated with pneumonia in humans. Interestingly, recent data illustrate the zoonotic potential of additional *Chlamydia* species such as *Chlamydia caviae*, *Chlamydia suis* and the more recently discovered species *Chlamydia gallinacea*.
We intend to give and overview on zoonotic *Chlamydia* infections focusing on clinical disease and diagnosis and will try to illustrate the importance of early diagnosis and awareness. Indeed, 'veterinary' chlamydial agents have received less attention by physicians. Human medicine should be more aware of the zoonotic potential of *Chlamydia* as there is accumulating evidence that these species are more abundant in animals than previously assumed. Also, recent data stress the need for a close collaboration between physicians, medical microbiologists, veterinarians and public health officials, as crucial information for source tracing, such as potential animal reservoirs can otherwise be missed.

S-23 Rectal shedding of *Chlamydia* suis in a pig farm without classical clinical signs of Chlamydiosis

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Background

Chlamydia (C.) suis is a well-known cause of fertility problems and conjunctivitis in pigs. The intestine, however, represents the main reservoir. Little is known about rectal shedding and transfer between pigs. The objective of the study was to illustrate both rectal excretion and infection dynamics in a piglet producing farm without clinical chlamydial history and vaginal/cervical shedding, respective.

Method

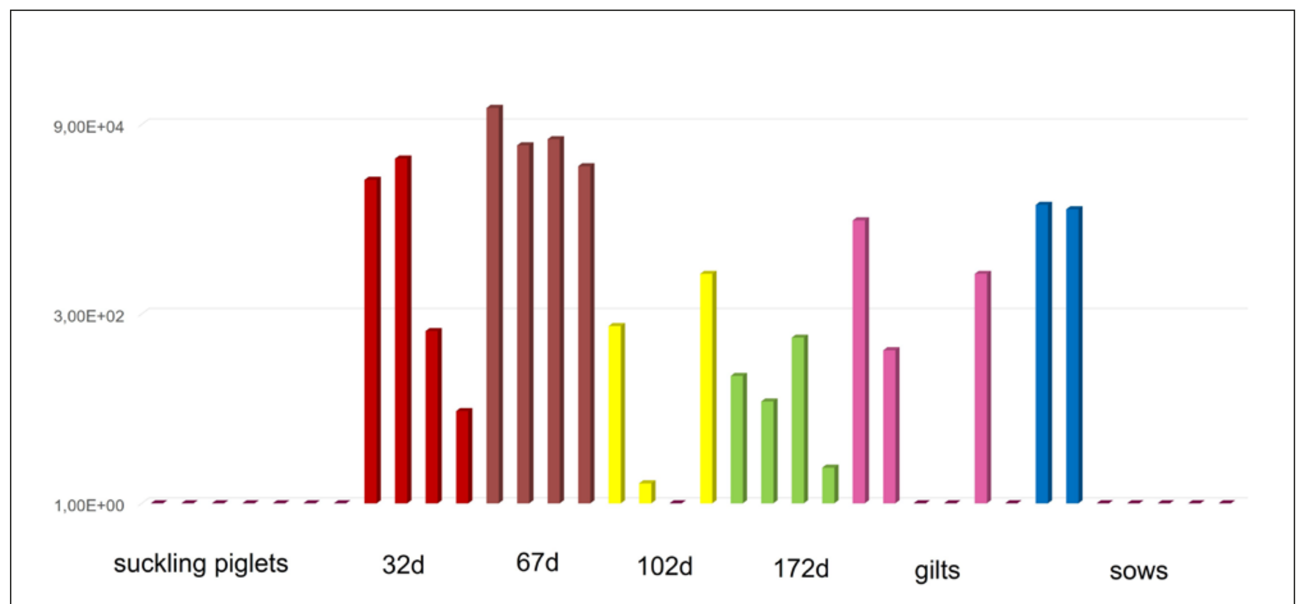
A cross-sectional study was done by taking rectal swabs from sows, own-reared gilts, suckling and nursery piglets at a single timepoint. In addition, rectal excretion of one sow and her offspring (5 of 12) was monitored longitudinally at regular intervals over a period of three months after birth. All samples were analysed by quantitative PCR for the presence of *C.suis*-specific DNA.

Results

Only 29% of the sows and 30% of the gilts shed *C.suis* rectally. While rectal excretion of *C.suis* by suckling piglets was not seen, excretion by nursery piglets was present (100%) and increased in quantities with age (104/g faeces in week 5; 105/g faeces in week 11). Thus, excretion was highest, when pig density was highest. The longitudinal study showed intermittent low-level *C.suis* shedding of the sow during suckling period. First positive faeces was detected 16 days after birth (101/g faeces) in one piglet, while high-level faecal shedding was found three weeks after weaning in all piglets (104/g faeces).

Conclusions

Even if *C.suis* is not causing clinical signs in a herd, it can still be found in faeces, most likely in nursery and fattening pigs. Intestinal colonisation in suckling piglets appears to be a slow process. In contrast, colonisation after mixing litters is much faster and seems unavoidable. The faecal bacterial load seems to be dependent on pig density. This might be a valuable information, when it comes to the question of reduction of chlamydial loads in farms with typical chlamydia-associated diseases.



S-24 *Chlamydia* serology in chickens using a bead-based peptide suspension array

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Background
Chlamydia gallinacea and *Chlamydia psittaci* are chlamydial species that can be detected in chickens and differ in epidemiology, clinical relevance and zoonotic potential. Because of these differences, it is important to have diagnostic tools available that allow detection and distinction. Current diagnostic tests in chickens rely on molecular detection, but discriminatory serologic tests are lacking. Therefore, we investigated the possibility to develop a serological test that can differentiate between antibodies against different *Chlamydia* species in chicken serum.

Method
A bead-based suspension array for antibody detection in chicken sera was developed using peptides as antigens. Published peptide sequences derived from *C. psittaci* and *C. gallinacea*, and from two genetically closely related species (resp. *Chlamydia abortus* and *Chlamydia avium*) were used. Sera from experimentally infected mice (kindly donated by B. Kaltenboeck and C. Schnee), experimentally infected chickens (*C. gallinacea* or *C. psittaci*), and 120 field chicken sera (from avian influenza monitoring) were tested.

Results
Results obtained with mice sera confirmed that *Chlamydia* derived peptides can be used as antigens in a beadbased suspension array for detection of antibodies against *Chlamydia*. In sera from experimentally infected chickens, antibodies against *C. psittaci* and *C. gallinacea* were detected specifically. In field sera, subsets of *Chlamydia* derived peptides were recognized: about two-thirds of the layer sera that were seropositive in the suspension array recognized *C. gallinacea* derived peptides.

Conclusions
Current results show that *Chlamydia* derived peptides can be used as antigens in a bead-based suspension array for detection of serum antibodies against *Chlamydia* in chickens. Results with field sera confirm the published high prevalence of *C. gallinacea* in layers. Signals against peptides derived from other *Chlamydia* species are yet difficult to interpret as limited (*C. psittaci*) or no (*C. abortus, C. avium*) sera from experimentally infected chickens were available for evaluation; this will require additional efforts in the future.

M-25 **KrobsPro: a collaborative teaching project**

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The objective of the KrobsPro joint project was to create a website dedicated to the teaching of microbiology. This project was the fruit of a call for proposals for the development of bilateral educational projects launched within the framework of the Université Libre de Bruxelles - University of Lausanne (ULB-UNIL) privileged partnership.
The project took the form of two workshops (organized successively in Lausanne in September 2021 and in Brussels in April 2022). A total of 22 students (11 from ULB and 11 from UNIL) and four teachers participated. The students, divided by Belgian-Swiss mixed pairs, worked on the writing of web pages. During the two weeks, key concepts in the field of microbiology, infectious diseases and their repercussions on public health were explored through various activities including ex-cathedra courses, role-playing games, exchanges with clinicians, and lab practice. These key concepts, as well as a personal research work done during dedicated time slots have allowed students to create their web pages. Each page was then presented to the entire group for comments and discussion before the final version was released. A total of 96 "pathogen" pages were created, as well as 11 "diagnostic methods", 11 "syndrome" and 13 "famous microbiologists" pages.
This project gave a unique opportunity for students to interact and establish long lasting connections. The Krobspro site they created, both playful and didactic, fully fulfills its purpose to complement and illustrate the ex-cathedrae courses offered to medical and public health students of both UNIL and ULB.

M-26 The Dark and Bright side of the force: teaching the importance of microbes from school to University

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Science skepticism and the poor transfer of scientific knowledge beyond academic circles represent barriers to overcome to tackle more effectively upcoming societal challenges such as the climate and biodiversity crises, as well as for fighting future pandemics. Therefore, dissemination of scientific knowledge beyond peer-communication should become a priority for all scientists. However, lay communication is not yet a component of the typical curriculum in university training. This is acutely evident in the case of microbiology. Microbiology is an essential element of our everyday lives, and this is not only the consequence of the importance of some pathogenic microorganisms, but also due to the positive and important role played by microorganisms in processes related to human health, food production or waste recycling, to cite a few. Nevertheless, the wider public still attributes a negative connotation to microbes. The aim of this study was to implement service-learning as a pedagogical approach to increase microbiology literacy in society. Service-learning is a teaching and learning strategy that integrates community service and civic responsibility, fostering the dissemination of scientific knowledge. This approach is used to communicate about pressing societal issues surrounding microbes (antibiotic resistance, natural resources recycling, human microbiota, and food production among others) to an audience of pupils attending public schools. The activity is proposed in the final year of the biology bachelor curriculum. Its purpose is to allow university students to develop and practice their lay communication skills as a key competence to be acquired during their university education. With this pedagogical approach, we intend to raise awareness regarding about the importance of microbiology and its dual roles as harmful and beneficial for humans. We also wish to increase the connection between primary, secondary and tertiary educational institutions, and highlight the importance of educating a new generation of communication savvy professionals in biology.

M-27 KROBS and MyKROBS: two playful card games dedicated to lay communication about pathogenic microorganisms

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Recently, because of the SARS-CoV-2 pandemic, the general population became aware of the presence of potentially harmful microorganisms in its environment. However, people generally still have poor knowledge of the multiple microbes, which they are exposed to in their daily life, and of the preventive measures that they could adopt to avoid coming into contact with them. To increase the general population awareness of pathogenic microorganisms, we created KROBS and MyKROBS, two card games, intended for 2-4 players from the age of eight and developed in collaboration with a professional game designer and a talented illustrator.
The gameplay was designed so that players encounter diverse situations of everyday life and learn the safe or risky behaviors regarding exposure to pathogens, while having fun in playing. KROBS and MyKROBS present altogether 60 microbes including the medically most relevant ones as well as several emerging pathogens. Different colors of cards are used to illustrate various modes of transmission of microbes, for example by food, by droplets/aerosols, by contact with ticks, faeco-oral, zoonotic, through wounds or when travelling to far countries. Furthermore, special black cards illustrate life situations that increase or decrease the risk of infection (immunosuppression, pregnancy, probiotics intake ...). In addition to messages directly transmitted while playing, a QR code on each card gives access to a dedicated website providing precise and detailed information (reservoirs, infection risks, prevention opportunities, symptoms, treatments) about each microorganism presented in the games.
KROBS and MyKROBS were initially developed for lay communication purposes. However, they are also used at Lausanne University as complementary education tools following 10 hours of ex-cathedra classes in microbiology. A web-based questionnaire survey revealed a positive interest of medical students for these two card games and a valuable impact of this learning material on their knowledge and performance in microbiology.

S-25 Lay communication on the risk of tick-borne diseases: a comparative study of the Swiss media landscape from 2000 to 2020

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Global warming and other environmental changes have important impact on the ecology of ticks in Switzerland. These arthropods formerly present only in the plains are now found at altitudes up to 2000m and their activity season increased, ranging now from March to November. Ticks have three stages of development and each stage transition requires a blood meal. In addition, changes have occurred in the habits and life style of the Swiss population during the last decades and people are more and more frequently in contact with ticks, especially during outdoor leisure activities. Yet ticks are important vectors of pathogens, some of them well known like *Borrelia burgdorferi* or TBE virus, the agents of Lyme's disease and tick-borne encephalitis, some other still considered as emerging pathogens. Unsurprisingly, tick-borne diseases are in constant progression in Switzerland according to the Federal Office of Public Health (FOPH). Although generally asymptomatic or associated with mild and unspecific symptoms, these diseases can sometimes have serious consequences or even fatal outcome. In this context, it is very important to inform the population about the risks of tick bites, especially since several simple but effective measures exist to avoid being bitten during outdoor activities. Media play an important role in public risk perception and in popularizing or spreading preventive measures. We were thus interested in assessing quantitatively and qualitatively the media coverage of the risk of tick bites and tick-borne diseases in French-speaking Switzerland during the two last decades.
We used ARGUS DATA INSIGHTS, a comprehensive database regrouping over 650 French-speaking traditional Swiss media (print media, radio and TV channels), and we searched for publications related to ticks and to tick-borne infections between January 2000 and December 2020. After manual elimination of irrelevant articles and duplicates, we retrieved 90 articles that were closely analysed with regard to their content and to the prevention messages that they convey to the population.
We observed an increase in the number of publications dealing with tick-borne diseases in the past 10 years. This increase perfectly correlates with the one of tick bites declared to FOPH. We also noticed a seasonality in the number of publications that exactly corresponds to the seasonal activity of ticks.
In addition, our analysis also revealed that only 28% of the retrieved articles were considered as highly pertinent which implicated that more than 2/3 of the publications were not informative enough. Sixty percent of the articles were mentioning pathogens, disease symptoms and preventive measures, indicating an average coverage of these important topics.
To conclude, this study confirmed that lay communication through mass media on the topic of tick-borne infections is not perfectly adapted, neither by the number of publications nor by their content, to the challenges that these diseases might represent in the future.

S-26 From Koch to Prowazek: famous microbiologists in the field of intracellular bacteria

Tristan Born, Milad Duloo, Alix Coste, Olivier Vandenberg, Marie Hallin, Gilbert Greub and the KrobsPro team

Bruxelles & Lausanne

As part of a bilateral project between the Université libre de Bruxelles and the university of Lausanne, a website called KrobsPRO has been developed by medical student for medical students and any other healthcare workers. In this website, informations about various microbes and various infections are presented including intracellular bacteria such as typhus, spotted fever, cat-scratch disease, chlamydial infections, q fever, … In addition, we also present on the website krobspro.ch a few number of famous microbiologists are also presented. During the international meeting on intracellular bacteria, microbiologists who played a pivotal role in the discovery and/or the understanding of the biology and medical importance of some established intracellular bacteria will be presented.
First, one student will present the life and achievements of Robert Koch. Briefly, Robert Koch (Germany) was born in 1843 in Clausthal and died in 1910 in Baden-Baden. He discovered the bacteria that cause tuberculosis, cholera and anthrax and he is thus considered one of the founders of medical bacteriology. He was also the first to use agar plate to cultivate microbes in the laboratory and his assistant Julius Petri developed the famous Petri dish, still used today. Finally, Koch is at the origin of the four "Koch's postulates", used to establish the causal relationship between a microbe and a disease.
Then, a 2nd student will present the life of Stanislas von Prowazek (Czech). Noteworthy, Stanislas von Prowazek was born in 1875 and discovered the agent of typhus in 1913. He then died from typhus in 1915, while studying this obligate intracellular bacteria, after being infected in Istanbul by the agent of epidemic typhus, which was then named in his honour *"Rickettsia prowazekii"*. He also reported the inclusion (vacuoles) produced by *Chlamydia trachomatis,* the agent of trachoma.
On the krobspro.ch website, you will also listen about Helene Sparrow who also studied typhus and then extended her work on vaccines to various other major pathogens such as *Vibrio cholerae.* Albert Calmette and Charlotte Olivier, both active on another major pathogen corrupting the host cells at his own advantage, i.e. Mycobacterium tuberculosis, as well as about Willy Burgdorfer, Joseph Lister are also presented on the same website.
We hope that the website will be a useful source of information for the future generations of medical students and for all persons interested in microbiology and infectious diseases.

M-28 **Metabolic Plasticity and Host Cell Tropism in *Coxiella burnetii***

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Coxiella burnetii, the etiological agent of human Query (Q) fever is a zoonotic bacterial obligate intracellular parasite. Q fever generally presents as an acute febrile disease; however, infection can progress to severe illnesses, including endocarditis. Following infection, *C. burnetii* replicates exclusively within a host-derived compartment, the *Coxiella* Containing Vacuole (CCV), and while exhibiting tropism for phagocytic cells *C. burnetii* is capable of infecting a broad range of eukaryotic cell types. This amphotropic nature (i.e., the ability to infect a broad host range and/or cell types) of *C. burnetii* suggests the pathogen relies on metabolic plasticity to establish infection and replicate in nutritionally diverse intracellular environments. To test whether *C. burnetii* metabolic plasticity affects pathogen virulence, intracellular replication in physiologically distinct cell types was compared between a metabolically competent parental strain and a mutant (*Cb∆pckA*) unable to undergo gluconeogenesis. Both the parental strain and *Cb∆pckA* exhibited host cell-dependent infection phenotypes. Moreover, infection phenotypes correlated with intracellular levels of host glycolytic (glucose) and gluconeogenic (amino acid) substrate availability. Of note, manipulation of *C. burnetii* carbon metabolism by ectopic expression of glucose-6 phosphate dehydrogenase reduced pathogen fitness under glucose limiting conditions. Since eukaryotic host cell physiology and growth characteristics directly influence replication of intracellular parasites, the physiological state of the host cell has the potential to mask the impact of pathogen metabolic defects. As such, and because the nutritional environment directly impacts host cell physiology, the analysis was extended to investigate the response of *C. burnetii* replication in host cells cultivated in a novel physiological medium (Interstitial Fluid-modeled Medium (IFmM)), which better resembles the nutrient composition of mammalian interstitial fluid. An infection model based on IFmM allowed for improved resolution of the growth defect exhibited by *Cb∆pckA* in some but not all host cell types. Collectively, we connect *C. burnetii* metabolic plasticity with the bacterium's amphotropic nature, metabolic fitness, and virulence.

M-29 **Host genetics: impact on *Chlamydia* infection**

Prof. dr. Servaas A. Morré

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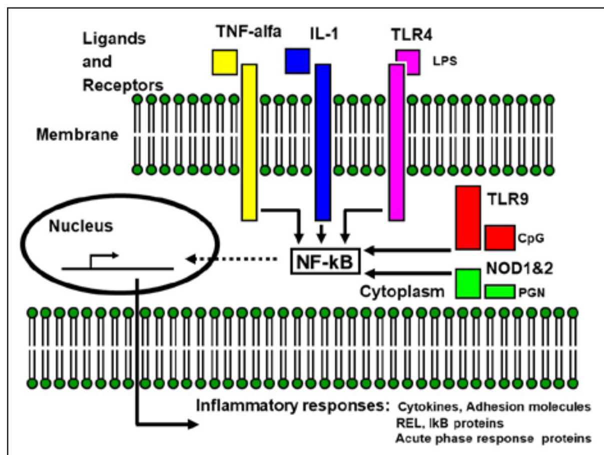
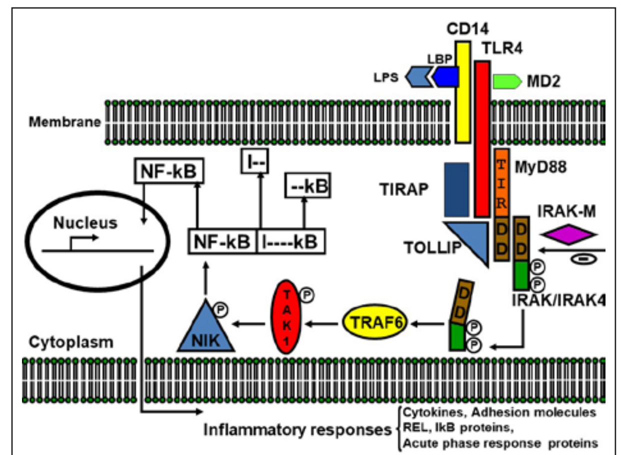
Striking inter-individual differences in the course of genital *C. trachomatis* infection are not only found in the development of symptoms and late complications. Also transmission of the pathogen during intercourse does not always occur, since not all partners of *C .trachomatis* positive persons appear to be infected. Ranges of transmission to partners differ from 65-76% in a group of symptomatic patients to 48% in asymptomatic patients. Another inter-individual difference in genital *C. trachomatis* infections is in clearance and persistence of infection. Only 45% of women who are asymptomatic clear the infection within one year. Difference in transmission, course of infection and development of sequelae cannot be ascribed to solely pathogen factors and environmental factors. Studies on the association between virulence of different chlamydia serovars and the course of a genital *C. trachomatis* infection did not yield reproducible and clinically applicable results. One of the major predictors for the clinical outcome and secondary sequelae of a *C. trachomatis* infection is the host genetic profile. Based on a twin study almost 40% contribution of host genetic factors to the clinical course of a chlamydial infection is estimated.
This narrative review provides an insight in the current knowledge of serology and host genetic factors (SNPs derived from host pathogen interaction pathways (See figures), murine knockout models, GWAS studies, etc) in *C. trachomatis* infections, TFI and time to pregnancy and their use as potential clinical biomarkers. The role of these two biomarkers in the prediction of TFI and time to pregnancy are addressed, and their role in future implementation of these two epidemiological and diagnostic tools is described.





S-27 Regulator of Actin-Based Motility (RoaM) Downregulates Actin Tail Formation by *Rickettsia rickettsii* and Is Negatively Selected in Mammalian Cell Culture

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Background

The etiological agent of Rocky Mountain spotted fever, *Rickettsia rickettsii*, induces the polymerization of actin filaments to propel the bacterium through the cytoplasm and spread to new host cells. Cell-to-cell spread via actin-based motility is considered a key virulence determinant for spotted fever group rickettsiae, as interruption of *sca2*, the gene directly responsible for actin polymerization, has been shown to reduce fever in guinea pigs. However, little is known about how, or if, motility is regulated by the bacterium itself.

Method

We isolated a hyper-spreading variant of *R. rickettsii* Sheila Smith and identified *A1G_06520* (*roaM* [regulator of actin-based motility]) as a negative regulator of actin tail formation by generating a recombinant knockout strain, and strains producing epitope-tagged RoaM. Immunofluorescence assays were used to quantify changes to motility. Reverse transcription-quantitative PCR (RT-qPCR) was used to assess the potential effect of RoaM on genes associated with motility. The generated strains assessed for changes in virulence using Guinea pigs.

Results

Disruption of RoaM significantly increased the number of actin tails compared to the wild-type strain but did not increase virulence in guinea pigs; however, overexpression of RoaM dramatically decreased the presence of actin tails and moderated fever response. Localization experiments suggest that RoaM is not secreted, while RT-qPCR data show that various levels of RoaM do not significantly affect the expression of the known rickettsial actin-regulating proteins *sca2*, *sca4*, and *rickA*. Serial passage experiments revealed strong negative selection of *roaM* in Vero 76 cells.

Conclusions

Taken together, the data suggest a previously unrecognized level of regulation of actin-based motility in spotted fever group rickettsiae. Although this gene is intact in many isolates of spotted fever, transitional, and ancestral group *Rickettsia* spp., it is often ablated in highly passaged laboratory strains. These findings are relevant to the interpretation of studies concerning virulence determinants of rickettsiae.

S-28 *Orientia tsutsugamushi* selectively stimulates the C-type lectin receptor Mincle and type 1-skewed proinflammatory immune responses

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Background

Orientia tsutsugamushi is an obligately intracellular bacterium and the etiological agent of scrub typhus. The lung is a major target organ of infection, displaying type 1-skewed proinflammatory responses. Lung injury and acute respiratory distress syndrome are common complications of severe scrub typhus; yet their underlying mechanisms remain unknown. In this study, we investigated whether the C-type lectin receptor (CLR) Mincle contributes to immune recognition and dysregulation.

Method

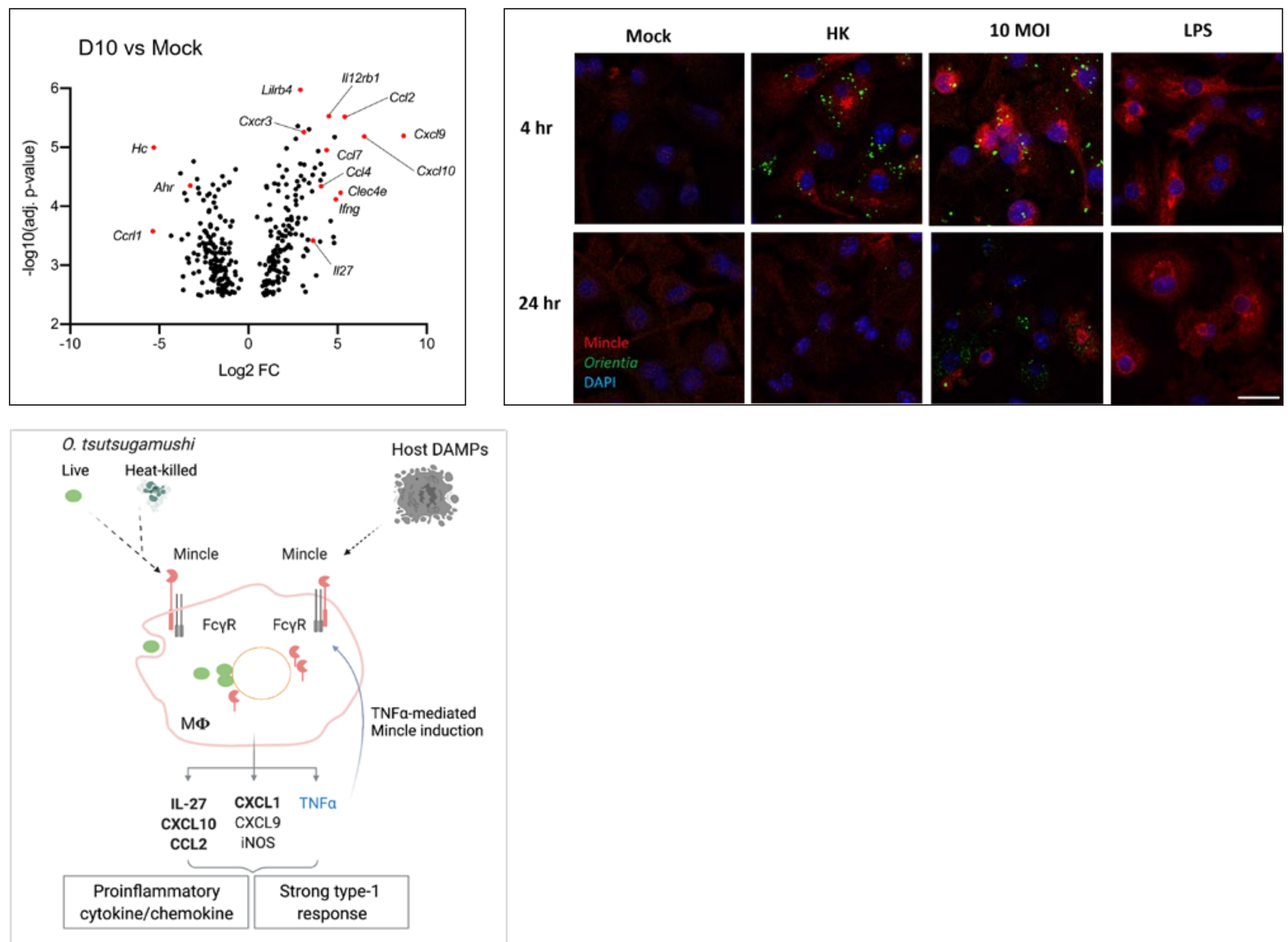
Following lethal infection in mice, we performed pulmonary differential expression analysis with NanoString, qRT-PCR, IFA, and WB analyses. We also infected primary macrophage cultures and used TNFa to explore Mincle-related responses.

Results

Of 671 genes examined, we found 312 significantly expressed genes at the terminal phase of disease. Mincle (*Clec4e*) was among the top 5 greatest up-regulated genes, accompanied with its signaling partners, type 1-skewing chemokines *Cxcr3*, *Ccr5*, and their ligands, as well as *Il27*. To validate a role of Mincle in scrub typhus, we exposed murine bone marrow-derived macrophages (MF) to live or inactivated *O. tsutsugamushi* and analyzed a panel of CLRs and proinflammatory markers via qRT-PCR. We found that while heat-killed bacteria stimulated transitory Mincle expression, live bacteria generated a sustained response in MF. Mincle protein levels were validated by indirect immunofluorescence and western blot. Notably, infection had limited impact on other tested CLRs. Sustained proinflammatory gene expression in MF (*Cxcl9, Ccl2, Ccl5, Nos2, Il27*) was induced by live, but not inactivated, bacteria.

Conclusions

Together, this study provides the first evidence for a selective activation of Mincle in sensing *O. tsutsugamushi* and suggests a potential role of Mincle- and IL-27-related pathways in host responses to severe infection. Additionally, it provides novel insight into innate immune recognition of this poorly studied bacterium.



M-30 **Selective fragmentation of the *trans*-Golgi apparatus by *Rickettsia rickettsii***

Ted Hackstadt

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Fragmentation of the Golgi apparatus is observed during a number of physiological processes including mitosis and apoptosis, but also occurs in pathological states such as neurodegenerative diseases and some infectious diseases. Virulent strains of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, induce selective fragmentation of the *trans*-Golgi network (TGN) soon after infection of host cells by secretion of the effector protein Rickettsial Ankyrin Repeat Protein 2 (RARP2). Remarkably, this fragmentation is pronounced for the *trans*-Golgi network but the *cis*-Golgi remains largely intact and appropriately localized. Thus *R. rickettsii* targets specifically the TGN and not the entire Golgi apparatus. Dispersal of the TGN is mediated by the secreted effector protein RARP2, a recently identified type IV secreted effector that is a member of the clan CD cysteine proteases. Site-directed mutagenesis of a predicted cysteine protease active site in RARP2 prevents TGN disruption. General protein transport to the cell surface is severely impacted in cells infected with virulent strains of *R. rickettsii*. Among the proteins showing decreased surface expression is the major histocompability complex 1 protein. Reduced surface expression of the MHC-1 due to impaired trafficking might thereby contribute to *R. rickettsii* evasion of immune surveillance. Recent studies implicate the SNARE protein syntaxin 5 in disruption of the TGN. In addition, we find that *rarP2* expression is regulated by previously unrecognized mechanisms. These findings suggest a novel manipulation of cellular organization by an obligate intracellular bacterium to determine interactions with the host cell.

M-31 Manipulation of host immune defense signaling, the key for intracytosolic colonization by pathogenic *Rickettsia* species

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Background

Rickettsia species are obligate intracellular bacteria that exhibit a range of virulence from harmless endosymbionts of arthropods to the etiologic agents of severe disease. One critical feature of these stealthy group of pathogens is their ability to manipulate the hostile cytosolic environment of the host to their benefits. However, the mechanisms of host innate immune defense evasion by pathogenic *Rickettsia* species remains to be elucidated.

Methods

Mechanisms of host defense evasion by *Rickettsia* species was evaluated using C57BL/6J mice and bone-marrow derived macrophages. The importance of IL-1 cytokines in limiting *Rickettsia* replication was evaluated using antibody-blocking and adaptive transfer experiments.

Results

We tested the hypothesis that pathogenic *Rickettsia* species evade immune responses in host defense cells, like macrophages, to replicate and disseminate. In this effort, we evaluated the cytosolic host defense responses between pathogenic *R. rickettsii* and *R. typhi* and non-pathogenic (*R. montanensis*) strains *in vivo and in vitro*. We showed that disease severity in wild-type (WT) C57BL/6J mice infected with *R. typhi* and *R. rickettsii*, but not with *R. montanensis*, correlated with levels of bacterial burden in the spleens, as well as the serum concentrations of pro-inflammatory cytokines (e.g., IL-1). Antibody-mediated neutralization experiments identified IL-1α as a key mediator to control mortality rates and bacterial burdens of *Rickettsia*-infected mice. We further determined the mechanism of the anti-rickettsial activities using bone-marrow-derived macrophages and found that *R. typhi* and *R. rickettsii*, but not *R. montanensis*, induced autophagy, and avoided autophagolysosomal destruction, while simultaneously eluded pro-IL-1α induction and benefited from a reduced IL-1α secretion, via a Caspase-11-Gsdmd-dependent mechanism, to facilitate intracytosolic replication.

Conclusion

In sum, we identified a previously unappreciated pathway by which pathogenic, but not non-pathogenic, *Rickettsia* preferentially target the non-canonical inflammasome-IL-1α signaling axis in macrophages, possibly via an autophagy-dependent mechanism, to support their replication and dissemination within the host.

S-29 New insights on the biogenesis of Coxiella-containing vacuoles revealed by phospholipid profiling

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Background
Coxiella burnetii is a class 3 pathogen responsible for the zoonosis Q fever, a debilitating disease with severe health and economic impact. Key to *Coxiella* virulence is the Dot/Icm-dependent secretion of bacterial effector proteins that coordinate the biogenesis of a large *Coxiella*-Containing vacuole (CCV) with autolysosomal features. Phosphoinositides (PIs) are emerging as targets for a growing number of bacterial effector proteins. These lipids are key players in eukaryotic cell homeostasis, define the identity of intracellular membranes and serve as regulators of eukaryotic signal transduction. Importantly, we have observed that perturbing the capacity of *Coxiella* to manipulate PI metabolism for CCVs biogenesis has *in vivo* relevance.

Method
Here we used automated microscopy and screening approaches to define the PI profile of CCVs and identify PI-interacting effector proteins (PIEs) regulating the biogenesis of CCVs.

Results
We observed that PI(3)P, PI(4)P and LBPA are actively enriched at CCVs in a Dot/Icm-dependent manner. Screening a sub-library of *Coxiella* transposon mutants in genes encoding candidate effector proteins we identified a new *Coxiella* effector protein, VicE (for Vacuole-inducing *Coxiella* Effector) as important for CCVs biogenesis and responsible for LBPA enrichment at CCVs. VicE specifically associates with LBPA-enriched large unilamellar vesicles (LUVs) and surprisingly, its ectopic expression in eukaryotic cells is sufficient to trigger the formation of a large vacuole that is reminiscent in morphology and composition to CCVs.

Conclusions
Our results further expand on *Coxiella* subversion of host lipid trafficking for CCV biogenesis and shed light on a new *Coxiella* effector involved in the manipulation of secretory membrane traffic during infections. Drawing a comprehensive map of the PI/*Coxiella* interactome that orchestrates CCVs biogenesis will help identifying key interaction hubs for the development of new, tailored antimicrobials.

S-30 The retropepsin-type protease APRc as a novel moonlighting immune evasion factor of Rickettsia

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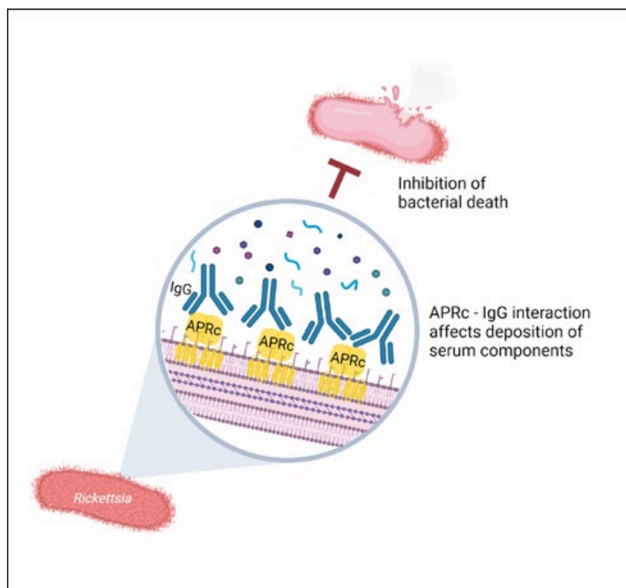
Background
Rickettsiae are obligate intracellular Gram-negative bacteria transmitted by arthropod vectors. Despite their reduced genomes, the function(s) of the majority of rickettsial proteins remains to be uncovered. APRc is a highly conserved retropepsin-type protease, suggested to act as a modulator of other rickettsial surface proteins with a role in adhesion/invasion. However, APRc's function(s) in bacterial pathogenesis and virulence remains unknown. This study demonstrates that APRc targets host serum components, combining non-immune immunoglobulin (Ig)-binding activity with resistance to complement-mediated killing.

Method
We have used different methodologies, ranging from whole-cell ELISA, ELISA assays, biolayer interferometry, pull-down and immunoprecipitation assays, Western and Far-Western blot to study APRc-Ig non-immune binding activity. Moreover, these studies were complemented with serum resistance assays and flow citomety analysis to evaluate the functional relevance of APRc-IgG interaction.

Results
We confirmed non-immune human IgG binding in extracts of different rickettsial species and intact bacteria. Our results revealed that the soluble domain of APRc is capable of binding to human (h), mouse, and rabbit IgG and different classes of human Ig (IgG, IgM, and IgA) in a concentration-dependent manner. APRc-hIgG interaction was confirmed with total hIgG and normal human serum. APRchIgG displayed a binding affinity in the micromolar range. We provided evidence of interaction preferentially through the Fab region and confirmed that binding is independent of catalytic activity. Mapping the APRc region responsible for binding revealed the segment between amino acids 157-166 as one of the interacting regions. Furthermore, we demonstrated that expression of the full-length protease in E. coli is sufficient to promote resistance to complement-mediated killing and that interaction with IgG contributes to serum resistance.

Conclusions
Our findings position APRc as a novel Ig-binding protein and a novel moonlighting immune evasion factor of Rickettsia, contributing to the arsenal of virulence factors utilized by these intracellular pathogens to aid in host colonization.



M-32 ***Chlamydia* vaccine initiative - what, who, how?**

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Background
An efficacious chlamydial vaccine will require identification of antigens and modalities that induce protective T cells. There is also a need for immune correlates of protection to aid vaccine efficacy testing.

Method
We collected behavioral, clinical, and microbiological data from a longitudinal cohort of *Chlamydia trachomatis* (CT) exposed women. Participants were evaluated for cervical and endometrial CT infection at enrollment, treated, and reassessed for CT infection and sexual risk behaviors at intervals over 12 months. We screened T cells from 30 CT+ women for IFNγ responses to 33 CT proteins by ELISPOT to identify potentially protective immunogens. Peripheral blood T cell subtypes were profiled by mass cytometry at enrollment and one month post treatment in women who remained uninfected during follow-up (FU-; n=12) and compared to those of women who developed reinfection (FU+; n=14). Comparisons were also made between responses of women who were negative for endometrial infection (non-ascenders; n= 15) to those of women who were positive (ascenders; n=13).

Results
Chlamydial protease-like activation factor (CPAF) was the most frequently recognized antigen by T cells and elicited the highest mean IFNγ+ T cell response that was CD4 dominant. When compared to FU+ women, FU- women exhibited higher central memory CD4 T cells, and an expanded CCR6+_CD4 T cell population, including CCR6+CCR4+CXCR3-Th17 and CCR6+CXCR3-CCR4-(CCR6+DN)_Th17-like subpopulations at enrollment. The CCR6+DN CD4 T cell population remained significantly elevated in FU-women at 1-month. Effector memory CD8 CCR6+DN_Tc17, and CD8 CXCR3+CCR6+Tc1_Tc17 and CD4_Treg cell populations were increased in the blood of CT non-ascenders vs ascenders.

Conclusions
CPAF is an immunoprevalent and immunodominant antigen in CT-exposed women, justifying its evaluation as a vaccine immunogen. CCR6+ CD4 central memory T cells may serve to protect from reinfection; CCR6+_CD8 T cells may aid in limiting infection to the cervix, and CD4_Tregs may limit immunopathology, respectively.

S-31 Effect of adjuvant on cellular and humoral immune responses to an experimental subcellular vaccine antigen in sheep

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Background

A desired goal of vaccines designed to protect against chlamydial infections is induction of cellular T-helper (Th)-1 type immunity with production of interferon-gamma (IFN-γ). We compared the cellular and humoral immune responses in sheep to a subcellular chlamydial outer membrane complex (COMC)-based vaccine antigen delivered in three adjuvants and correlated these with protective efficacy following challenge infection.

Method

Groups of 35 sheep were immunised with a single inoculation of COMC antigen formulated in two water-in-oil adjuvants (MontanideTM ISA 70 VG, MontanideTM ISA 61 VG) or saponin-derived QuilA. Sheep were mated and experimentally challenged with *Chlamydia abortus* at day 70 of gestation. To measure cellular responses, peripheral blood mononuclear cells (PBMC) were re-stimulated *in vitro* with COMC or killed *C. abortus* elementary bodies. The PBMC culture supernates were analysed for IFN-γ as an indicator of a Th1-type response and interleukin (IL)-4 as an indicator of a Th2-type response. Humoral immune responses to an outer membrane protein (OMP) were measured by ELISA. Efficacy was assessed as ability to prevent abortion.

Results

All three adjuvants induced COMC-specific cellular immune responses. In each case, IFN-γ was the dominant cytokine in the post-immunisation antigen-specific recall responses compared to IL-4. Of the three adjuvants, the MontanideTM ISA 70 VG stimulated the highest IFN-γ production, whereas QuilA-based formulation induced the poorest levels. Measurement of OMP-specific antibody responses revealed a significant up-regulation following experimental challenge across groups and no correlation with clinical outcome was observed. The ISA 70 VG/COMC and QuilA/COMC formulations provided the greatest and lowest protection levels respectively.

Conclusions

MontanideTM and saponin-based adjuvants elicit antigen-specific immune responses to the COMC antigen in sheep. The quality and magnitude of the cellular immune responses correlated with protection from abortion for the MontanideTM -adjuvanted vaccine groups. This demonstrates the crucial role of adjuvant selection in livestock vaccine optimisation.

S-32 Modified *Coxiella burnetii* polysaccharide vaccine protects guinea pigs from coxiellosis

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Background
Q fever, caused by the bacterium *Coxiella burnetii*, is an important zoonosis found world-wide. Infected, parturient vertebrate animals, especially goats, sheep and cattle, contaminate the environment when they give birth. There is an effective human vaccine used only in Australia (Q-VAX) but nowhere else due to difficulties associated with its use, including the risk of adverse reactions. A new human vaccine is needed.

Method
An experimental vaccine, prepared from the cell-wall polysaccharide (O-antigen) of virulent (phase 1) *C.burnetii*, strain Nine Mile, conjugated to a protein (tetanus toxoid), was used (with and without alum adjuvant) to vaccinate guinea pigs prior to intranasal challenge with *C.burnetii*. The guinea pig is an excellent animal model of acute Q fever.

Results
The vaccine protected guinea pigs from fever and weight loss on infectious challenge. The spleen, liver and kidney of vaccinated guinea pigs contained significantly fewer genomes of *C.burnetii* than those of unvaccinated control animals.

Conclusions
A conjugate, polysaccharide vaccine from the cell wall of phase 1 C.burnetii protected guinea pigs from coxiellosis and may prove to be useful in humans also.

S-33 Q fever post-vaccination hypersensitivity modeling reveals sex dimorphism and a less reactogenic vaccine candidate

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[1] Laboratory of Bacteriology, Intramural Research Program, National Institute of Allergy and Infectious Disease, National Institutes of Health, Hamilton, Montana, US

Background
Delayed-type hypersensitivity (DTH) responses to microbial vaccines and related components are a major roadblock for widespread licensing of whole cell vaccines such as that of Q fever. Q fever is a zoonotic disease caused by the intracellular bacterium *Coxiella burnetii*.

Method
Guinea pigs have been used to model late phase granulomatous DTH responses following Q fever vaccination; however, the early phase DTH response has not been recapitulated in this model. To address this, we developed a murine post Q fever vaccination DTH model which robustly represents the early phase DTH response observed in humans.

Results
In this model, female C57Bl/6J mice displayed the most intense early-phase DTH responses following sensitization compared to their male counterparts and other mouse strains. Immunologic responses were measured within the skin, draining lymph nodes, and serum following both sensitization and elicitation with Q fever whole cell vaccines (WCV). Local immunologic responses in the dermis were characterized by inflammation primarily involving neutrophils, macrophages, and T cells. Secondary lymphoid organ profiling revealed distinct immunological signatures following both sensitization and elicitation with a sex-based dichotomy in T cell phenotypes and antigen presenting cell numbers. Next, employing a guinea pig post-vaccination DTH model, we screened a genetically modified *C. burnetii* Dugway Δ*dot/icm* WCV, which demonstrated reduced reactogenicity and granulomatous DTH compared to wild-type WCVs.

Conclusions
The murine early-phase DTH model will be used for future vaccine screening and immunologic interrogation of this response. Beyond providing a novel post-Q fever vaccination DTH model that recapitulates early-phase DTH events, these data suggest that sex is a primary factor influencing the magnitude and composition of the ensuing response. Additionally, genetically modified WCVs appear to be viable vaccine candidates for Q fever. Together, this work will contribute to improved understanding of the post-vaccination DTH response and to the development of an improved Q fever vaccine.

S-34 Inclusion of Quillaria adjuvant in the Coxevac formulation skews *Coxiella burnetii*-induced inflammatory responses towards a sustained Th1-CD8+-mediated activation and increases protection in a goat vaccine-challenge experimental model

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Background
Q Fever is a zoonotic bacterial infection due to *Coxiella burnetii,* leading to reproductive disorders in ruminants. Coxevac® is the EMA-approved veterinary vaccine for protection of cattle and goats against Q Fever and the control of disease diffusion and transmission to humans. Since Coxevac® reduces bacterial shedding and clinical symptoms but does not prevent infection, novel vaccine formulations are needed to overcome this problem.

Method
In this study, a goat vaccination-challenge model was used to evaluate the impact of the saponin-based QuilA adjuvant on Coxevac® immunity.

Results
Compared to Coxevac® (Cox) only, vaccination including QuilA (Quil-A Cox) resulted in a transient increase in the mean body temperature and a protracted serum IgG production. None of the vaccine formulations affected the frequency of peripheral CD4+ and CD8+ T-lymphocytes, though each triggered a different *in vitro* recall IFNγ T-cell response. While Quil-A Cox activated antigen-specific IFNγ production after both prime and boost dose, Cox only triggered IFNγ production after the boost. Upon challenge*,* the Quil-A Cox group showed a stronger systemic immune response reflected in a higher magnitude of total IgG, an increase in circulating CD8+ T-cells and a biphasic antigen-specific IFNγ release compared to the Cox group. In spleen, higher levels of *CD8+, NRC1* and *IFNγ* transcripts were detected in the Quil-A Cox group as opposed to the challenged control group. The latter showed highly expressed pro-inflammatory cytokines, such as *IL1B* and *IL17A* in spleens and *IL6* in respiratory lymph nodes. An intriguing expression was observed for γδ T cells, which were of *TBX21*- and *SOX4*-types in the Quil-A Cox and challenged control group, respectively. Hence, QuilA adjuvant induced a moderate but increased protection efficacy.

Conclusions
Overall, the QuilA adjuvant enhanced adaptive immune responses triggered by Coxevac® in *C. burnetii*challenged goats, resulting in a stronger IgG production and a Th1-type CD8+ cell mediated response.

M-33 Transforming chlamydia using the Group II intron approach: strengths, weaknesses, and applications

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The ability to genetically modify bacteria is key for discerning how bacteria grow, replicate, cope with stress, and cause disease. While the list of genetic tools available for manipulating obligate intracellular bacteria has expanded in recent years, the approaches remain challenging to employ and applicability differs across species. The group II intron mutagenesis approach utilizes a targetable intron encoding a selection marker to construct site-specific insertions with mutants isolated using antibiotic selection. Two different group II introns have been developed for insertional mutagenesis, Ll.LtrB [commonly referred to as TargeTron] and EcI5, with Ll.LtrB the most widely utilized. Ll.LtrB has been employed in a wide variety of Gram positive and Gram negative bacteria including *Chlamydia* spp., *Ehrlichia chaffeensis* (transient mutant survival), and *Rickettsia rickettsii*. A general overview of the methodology and current platforms as they apply to obligates, with a chlamydial emphasis, will be provided. Limitations of the group II introns (Ll.LtrB/EcI5) focusing on unpublished work from our group will also be discussed. Finally, biological insights obtained through the use of group II intron mutagenesis in *Chlamydia* spp. by our group and others will be highlighted.

M-34 **Inducible knockdowns in *Chlamydia* using CRISPR interference**

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In evolving to obligate intracellular dependence, *Chlamydia* has streamlined its genomic size and content. As such, it is likely that a large proportion of its genes are essential and, therefore, refractory to disruption using standard genetic approaches. To address this limitation, we have developed an inducible knockdown system for *Chlamydia* using CRISPR interference (CRISPRi). This system is predicated on the ability of an inactive Cas (i.e. dCas) isoform to recognize and bind a genomic locus using a guide RNA and to sterically hinder RNA polymerase function at the promoter binding or elongation steps. Here, I will describe the development of vectors for CRISPRi in *Chlamydia*, their uses and advantages over other conditional knockout strategies, and other advancements in this area. Published and unpublished examples will be presented to illustrate the utility of the system to dissect chlamydial biology and the function of essential genes in this unique and unusual pathogen.

S-35 Transposon mutagenesis of *Chlamydia trachomatis*

Colette O'Neill [1], **Rachel Skilton** [1], **Jade Forster** [2], **David Cleary** [1], **Sarah Pearson** [3], **David Lampe** [4], **Nicholas Thomson** [5], **Ian N. Clarke** [1]

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Background

Chlamydia trachomatis is a human pathogen that causes long-term complications in women. In this study, a transposon mutagenesis approach was developed for *C.trachomatis* using a self-replicating vector to deliver the transposon-transposase cassette, enabling inducible transposon mutagenesis of the chromosome with the ultimate aim of identifying gene function.

Method

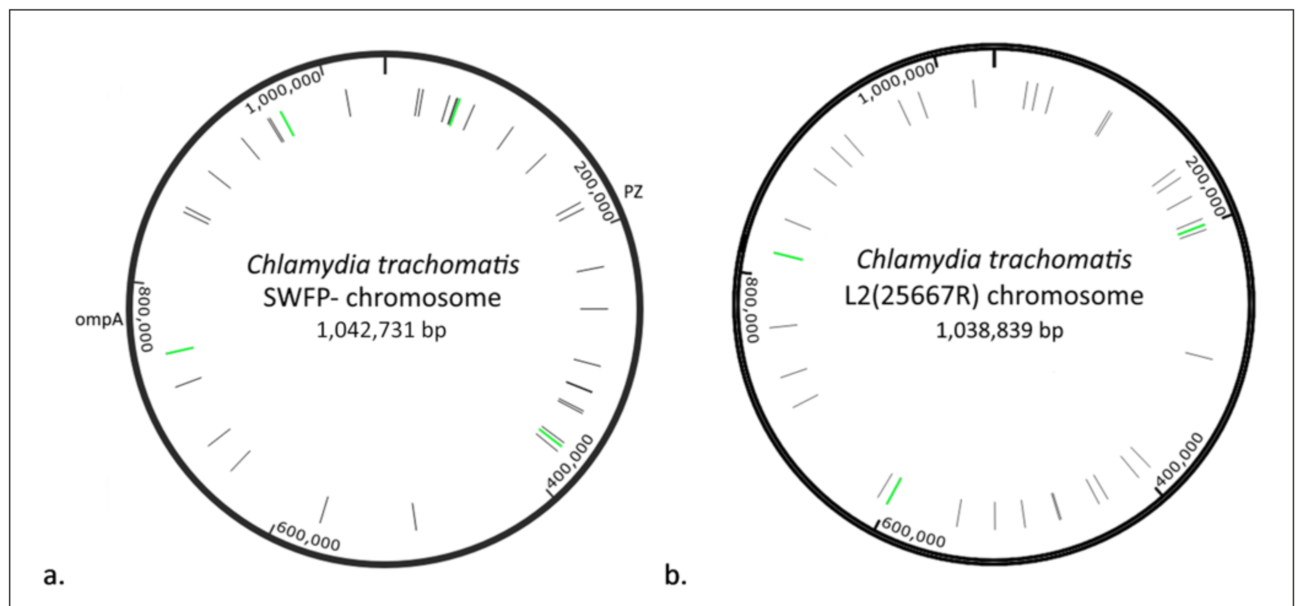
The low transformation efficiency of *C.trachomatis* necessitated the design of a self-replicating vector carrying the transposon mutagenesis cassette (Himar-1 transposon, a hyperactive transposase gene under inducible control of the *tet* promoter and a riboswitch). *Chlamydia* (L2(25667R) and SWFP-) transformed with this vector (pSW2-RiboA-C9Q) were induced at 24 hours post-infection, and harvested at 48 hours p.i. Sequencing libraries were prepared from DNA extracted from these mutant pools, and long- and short-read sequencing methods were used to identify chromosomal transposon inserts.

Results

DNA sequencing libraries were initially analysed using a MinION as a low-throughput screen of mutant libraries, providing 'proof of concept': 34 and 36 insertions were identified in L2 and SWFP- respectively. Passage of mutant pools enabled expansion of some mutants, suggestive of permissive gene disruption. Mutants lost upon passage may be due to disruption of essential genes, but further investigation is needed. High throughput shortread sequencing was recently performed to confirm whether saturation mutagenesis has been achieved; this will inform on which genes are essential in vitro.

Conclusions

This study generated the first stable self-replicating chlamydial transposon delivery vector. We identifed 36 and 34 insertions in SWFP- and L2(25667R) chromosomes in a single induction experiment using long-read sequencing; the use of high throughput sequencing will undoubtedly reveal many more. This study provides an efficient method for assaying *C.trachomatis* gene function and will enable the identification of the essential gene set of *C.trachomatis*. Four other chlamydial species have been successfully transformed by other researchers, so this transposon mutagenesis approach could easily be adapted for these.



S-36 **Possibilities and limitations of competence induction in *Chlamydia suis***

Hanna Marti [1], **Kensuke Shima** [2], **Siegrid Pätzmann** [2], **Jan Rupp** [2], **Steve J. Charette** [3], **Nicole Borel** [1]

[1] Institute of Veterinary Pathology, University of Zurich, Zurich, Switzerland [2] Department of Infectious Diseases and Microbiology, University of Luebeck, Luebeck, Germany [3] Department of biochemistry, microbiology and bioinformatics, Laval University, Quebec, Canada

Background

The obligate intracellular bacterial family of the *Chlamydiaceae* poses challenges for the field of molecular genetics. Competence is usually induced with the help of calcium chloride, using shuttle vectors for transformation. Apart from the construct of interest, these vectors generally contain the genetic backbone of the cryptic chlamydial plasmid. While this transformation method has been effective for many *Chlamydiceae* species such as *C. trachomatis* and *C. pneumoniae,* there is no existing protocol for *C. suis,* a chlamydial species infecting domestic pigs and wild boar. *C. suis* is the only chlamydial species that has naturally acquired homotypic antibiotic (tetracycline) resistance integrating into its chromosome. The aims of this study were to a) establish a transformation system for *C. suis* and b) to test whether *C. suis* is able to take up pRAS3-3432, a plasmid originating from the fish pathogen *Aeromonas salmonicida ssp. salmonicida,* which shares a high nucleotide identity with the plasmid that was naturally integrated into some *C. suis* strains.

Method

Our successfully established transformation protocol for *C. suis* included the use of calcium chloride. Briefly, we incubated the shuttle vector together with *C. suis* for 1h on ice, followed by heat shock, 20 min of incubation with trypsinized cells, and subsequent seeding onto 6-well plates. Passages in selective antibiotics were performed every 36-72h.

Results

Previously established chlamydial transformation protocols were not suitable for *C. suis*. We performed optimization experiments identifying optimal calcium chloride concentrations as well as the necessity of ice incubation and subsequent heat shock. Transformation experiments with pRAS3-3432 are currently ongoing.

Conclusions

Here, we describe an optimized protocol for successful transformation of *C. suis*. Currently, we are testing whether pRAS3-3432 is introduced into *C. suis* by competence induction.

M-35 **The microbiome of ticks: an overview**

Aránzazu Portillo

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Hard ticks are recognized as ones of the most important vectors of infectious diseases in the industrialized world. Their geographic distribution is expanding, making tick-borne diseases a growing threat to animal and human health. In clinical practice, the diagnosis of tick-borne diseases is a challenge. Most microorganisms transmitted by ticks cannot be isolated unless using cell culture, which is limited to specialized laboratories. The diagnosis is often made by serologic tests, but cross-reactions are common, and detection of antibodies depends on the time of sampling collection. During decades, the identification of microbes associated with ticks has been mainly based on using molecular biology methods (PCR and sequencing). More recently, new sequencing technologies and bioinformatics analysis of accumulated data have demonstrated to be a useful tool for diagnosis. In 2011, next generation sequencing techniques were applied for the first time to identify microbial communities related to ticks. But the term microbiome does not only encompass the genomes of microorganisms. The microbiome designates the community of microorganisms (bacteria, archaea, fungi, algae, eukarya and/or viruses), their genes, genomes, metabolites and functions within a specific niche, taking into account their interactions (environment-microbe, microbe-microbe and microbe-host) and changes over time. This holistic concept has emerged based on the application of multi-omics technologies (culturomics, metagenomics, metataxonomics, metatranscriptomics, metaproteomics, metabolomics…) combined with clinical, epidemiological and/or environmental metadata. Integrated meta-omics approaches to characterize the tick microbiome, and its function, are necessary to understand the extent to which the microbiome composition, modeled by the habitat and fauna, influences in the life cycle of ticks and in their capacity to acquire and transmit pathogens. Tick microbiome studies under a 'One Health' prism are promising to develop tick control strategies, to unravel interactions among tick-associated microorganisms, to discover new uncultured ones and subsequently, to improve the diagnosis of tick-borne diseases.

M-36 A Novel Flow Cytometric Approach for the Quantification and Quality Control of Chlamydia trachomatis Preparations

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Background
Chlamydia trachomatis is an obligate intracellular pathogenic bacterium with a biphasic developmental cycle manifesting two distinct morphological forms: infectious elementary bodies (EBs) and replicative intracellular reticulate bodies (RBs). Current standard protocols for quantification of the isolates assess infectious particles by titering inclusion-forming units, using permissive cell lines, and analyzing via immunofluorescence. Enumerationof total particle counts is achieved by counting labeled EBs/RBs using a fluorescence microscope.

Method
For a better assessment of *C. trachomatis* preparations, we developed a simple and time-saving flow cytometrybased workflow for quantifying small particles, such as EBs with a size of 300 nm. This included optimization of gain and threshold settings with the addition of a neutral density filter for small-particle discrimination. The nucleic acid dye SYBR® Green I (SGI) was used together with propidium iodide and 5(6)-carboxyfluorescein diacetate to enumerate and discriminate between live and dead bacteria.

Results
We found no significant differences between the direct particle count of SGI-stained *C. trachomatis* preparations measured by microscopy or flow cytometry ($p > 0.05$). Furthermore, we completed our results by introducing a cell culture-independent viability assay. Our measurements showed very good reproducibility and comparability to the existing state-of-the-art methods.

Conclusions
In summary, the evaluation of *C. trachomatis* preparations by flow cytometry is a fast as well as reliable method and thus facilitates an improved assessment of the quality of *C. trachomatis* preparations for downstream applications.

S-37* Identification of *Anaplasma* species in wildlife hosts in the Kruger National Park and surrounding game reserves using a bacterial microbiome approach

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Background

The rapid advancement of next-generation sequencing technologies has led to the discovery of many novel sequences ascribed to the genus *Anaplasma*, with nearly 20 new species being proposed since the last formal organization of the genus. Most of the 16S rRNA gene surveys for *Anaplasma* were conducted on cattle and to a lesser extent on rodents, dogs, and ticks. Little is known about the occurrence, diversity, or impact of *Anaplasma* species circulating in wildlife species.

Method

Therefore, we conducted a 16S rRNA gene survey with the goal of identifying *Anaplasma* species in a variety of wildlife species in the Kruger National Park and neighbouring game reserves, using an unbiased 16S rRNA genemicrobiome approach.

Results

An *Anaplasma*-genus specific qPCR assay revealed the presence of *Anaplasma* species in 70.0% (21/30) of African buffalo, 86.7% (26/30) of impala, 36.7% (11/30) of greater kudu, 3.2% (1/31) of African wild dog, 40.6% (13/32) of Burchell's zebra, 43.3% (13/30) of warthog, 22.6% (7/31) of spotted hyena, 40.0% (12/30) of leopard, 17.6% (6/34) of lion, 16.7% (5/30) of African elephant and 8.6% (3/35) of white rhinoceros samples. Microbiome sequencing data from the *Anaplasma*-positive samples revealed genotypes that phylogenetically group with known and previously published *Anaplasma* sequences, as well as novel *Anaplasma* genotypes.

Conclusions

Our preliminary results reveal a greater genetic diversity of *Anaplasma* species circulating in wildlife species than currently classified within the genus *Anaplasma*, suggesting potential for transmission to livestock or companion animals. Our findings highlight the need for genetic and genome sequencing of putative species for correct classification and further assessing occurrence in livestock and companion animals.

*Student paper

S-38 The bacterial microbiome of *rhipicephalus* sanguineus ticks in the mnisi community, South Africa

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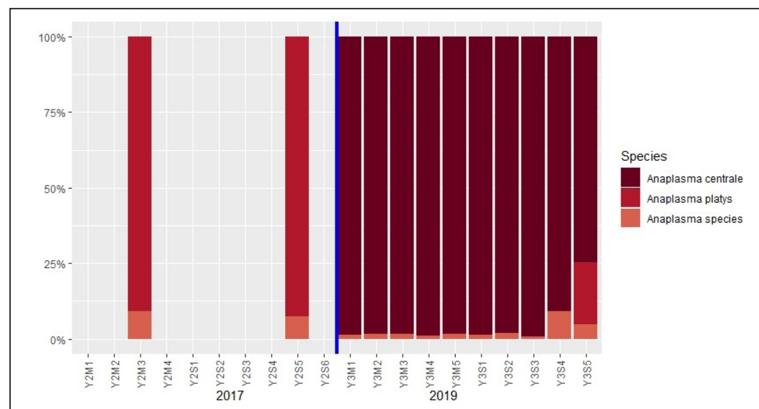
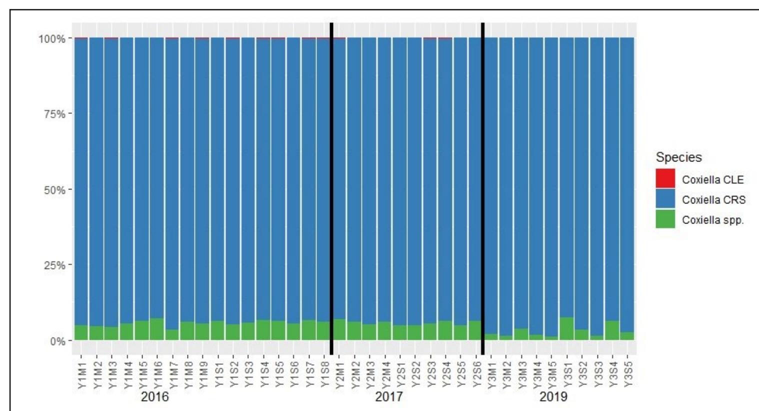
Background
Many emerging communicable diseases amongst humans can be ascribed to zoonotic pathogens arising from animals. *Rhipicephalus sanguineus* ticks are ideal vectors of zoonotic pathogens, and although capable of parasitizing most vertebrates they prefer dogs, and are thus prevalent on dogs, particularly in rural resource poor communities. The Mnisi community in Mpumalanga, South Africa is one such rural, impoverished community. The Mnisi community sits at the wildlife-livestock-human interface where humans are at risk of infection with various tick-borne zoonotic pathogens. The aim of this study was to characterize the bacterial microbiome of *R. sanguineus* ticks collected from dogs in the Mnisi community.

Method
To achieve this, we analysed the bacterial microbiome of ticks sampled from community dogs over three nonconsecutive years. After collection, ticks were kept in a humidity and temperature controlled chamber for two days to digest their blood meal. A total of 10 dogs were sampled in 2016, 7 dogs in 2017 and 6 dogs in 2019. From each dog, ten male *R. sanguineus* ticks were collected, surface sterilized and dissected. Separate pools were made from midguts (MG) and salivary glands (SG) from all 10 ticks. We processed 9 MG pools and 9 SG pools from 2016, 7 MG and 7 SG pools from 2017 plus 5 MG and 5 SG pools from 2019. Genomic DNA was extracted and amplified using universal 16S rDNA barcoded primers. Samples were then sequenced using Pacific Bioscience's circular consensus sequencing strategy.

Results
Our study detected *Anaplasma platys* from two samples in 2017 (2.53%) and one sample in 2019 (1.05%). *Anaplasma centrale* was detected in all 2019 samples (17.80%). *Coxiella*-like endosymbionts were detected in all samples from 2016 (74.54%), 2017 (84.29%) and 2019 (42.78%).

Conclusions
Our study highlights the need for further research into the role that *R. sanguineus* ticks play as a vector of bacterial pathogens.





M-37 Intracellular trafficking of *Orientia tsutsugamushi*

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Background

Orientia tsutsugamushi (Ot) is an obligate intracellular alpha-proteobacterium in the order Rickettsiales that causes the life-threatening vector-borne human disease scrub typhus. Unlike many intracellular bacteria that reside within membrane-bound vacuoles, it escapes from the endo-lysosomal pathways shortly after infection and replicates directly in the host cell cytoplasm. Ot has been shown to use microtubules to move within host cells, rather than actin filaments that are used by other Rickettsiaceae. Whilst Ot trafficking along microtubules is known to be dynein dependent, the molecular basis of this interaction was not known.

Method

Here, we show that Ot uses the surface autotransporter ScaC to traffic along microtubules using the dynein adaptor molecules BicD1 and BicD2. This interaction is essential for Ot relocation to the perinuclear region after infection, and for bacterial replication. We use biochemistry approaches to identify the binding regions in ScaC and BicD2 and cell biology approaches to demonstrate the impact of perturbations in ScaC and BicD1/2 on Ot position and replication.

Results

Ot uses its surface autotransporter proteins ScaC to exploit microtubule trafficking via dynein adapter proteins BicD1 and BicD2. This intracellular motility is required for bacterial replication.

Conclusion

Rickettsiaceae lack flagella genes and some species exploit host cell machinery to relocate to specific areas of the cell or to traffic into adjacent cells. Ot uses a previously poorly characterized microtubule-mediated process to traffic to the perinuclear region where it undergoes growth and replication.

M-38 **Formation of a pathogen vacuole according to *Legionella pneumophila***

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Legionella pneumophila is an amoeba-resistant, environmental bacterium and the cause of the severe pneumonia "Legionnaires' disease". The facultative intracellular pathogen creates in amoebae and macrophages a unique membrane-bound compartment termed the "*Legionella*-containing vacuole" (LCV). LCV formation requires the bacterial Icm/Dot type IV secretion system, which translocates more than 330 different "effector proteins" into host cells, where they subvert pivotal processes. LCVs restrict fusion with lysosomes, but communicate with the endosomal, secretory, and retrograde vesicle trafficking pathways, and they intimately interact with the endoplasmic reticulum (ER) through membrane contact sites (MCS).
A hallmark of LCV formation is the phosphoinositide lipid conversion from endosomal PtdIns(3)*P* to secretory PtdIns(4)*P*. Proteomics of purified, intact LCVs revealed host factors presumably involved in LCV formation, including small and large GTPases and MCS components. Indeed, *L. pneumophila* subverts the small GTPase Ran and the large GTPase atlastin 3 (Atl3)/Sey1, a fusion GTPase which promotes ER tubule dynamics and lipid droplet biogenesis. *Dictyostelium discoideum* amoebae lacking the single atlastin orthologue *sey1* are viable and represent a powerful model to study the role of the large GTPase in the context of *L. pneumophila* infection. Recent progress on the role of Sey1 and MCS components for LCV formation will be discussed.

S-39 Targeted mutagenesis broadly applicable for Ehrlichia and Anaplasma species in diverse research applications, including in creating gene deletions, fusion proteins, and in studies involving mRNA and protein synthesis knockdown

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Background

Tick-borne diseases resulting from obligate intracellular Anaplasmataceae family pathogens of the genera *Anaplasma* and *Ehrlichia* continue emerging as a major public health concern. Despite a few recent advances, genetic manipulations continue to hinder research in defining the virulence determinants of these pathogens. We recently reported the development of targeted mutagenesis to disrupt several genes in *Ehrlichia chaffeensis*; additionally, we recently reported a targeted gene deletion mutation in *Anaplasma marginale* aiding in defining virulence and vaccine development (Wang *et al*., Sci. Rep., 2017 and Hove *et al*. PLOS Pathog., 2022).

Method

We extended targeted mutagenesis to create C-terminal protein tags on several *E. chaffeensis* expressed proteins. In addition, a novel antisense mutation was generated.

Results

A tagged mutation producing green fluorescent protein aided in validating the translocation of a previously defined *E. chaffeensis* Type 4 Secretion System effector; Etf1 from *Ehrlichia* containing vacuoles (ECVs, also known as morula). Similarly, hemagglutinin tagged mutations have aided in studies to define *E. chaffeensis* DNA binding proteins (DBPs) interacting with numerous bacterial gene promoters. A novel antisense mutation was also created to synthesize a short segment of complementary RNA from p28-Omp 19 gene coding sequence. The antisense mutation caused significant reduction in the p28-Omp 19 mRNA and protein expression *in vitro.* The knockdown in the p28-Omp 19 gene expression in the mutant was compensated with augmented transcription from two p28-Omp 19 gene parologs: p28-Omp 14 and p28-Omp 20.

Conclusions

The data demonstrate that targeted mutagenesis in rickettsiales has diverse applications, such as investigating *in vivo* virulence and pathogenesis, vaccine development, bacterial effector proteins, DBPs, and gene expression knockdown studies from essential genes. As the methods are broadly applicable, we anticipate that the studies have transformational impact in advancing our understanding of several important tick-borne rickettsial diseases in people, companion animals and agricultural animals.

S-40* The Chlamydia trachomatis IncM protein interferes with host cell centrosome positioning and modulates inclusion structure

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Background
Chlamydia trachomatis manipulates host cells by delivering into them several effector proteins. Among these are a group of transmembrane proteins (Incs), which insert at the membrane of the vacuole (inclusion) that encloses this obligate intracellular pathogen. We previously showed that *C. trachomatis* IncM interacts with a host centrosomal protein. To understand the function of IncM during infection, we further characterized a IncM-deficient *C. trachomatis* strain.

Method
HeLa, hTERT-RPE1 or ARPE-19 cells were infected with *C. trachomatis* wild-type, IncM-deficient, IncM-deficient carrying a plasmid overproducing IncM (complemented strain) or plasmids encoding truncated versions of IncM or IncM homologues. The infected cells were analyzed by immunofluorescence microscopy for host cell multinucleation, centrosome positioning, and inclusion positioning and morphology. Infected cells were also analyzed after incubation with cytoskeleton-disrupting drugs.

Results
Cells infected by IncM-deficient *C. trachomatis* showed less multinucleation than cells infected by the wild-type or complemented strains. IncM homologues from other *C. trachomatis* strains and *Chlamydia* species also complemented this IncM-dependent defect. The ability of IncM to induce multinucleation seemed to correlate with its localization at the inclusion membrane and required its two larger regions predicted to be exposed to the host cell cytosol. Moreover, by comparison to cells infected by the wild-type strain, in cells infected by IncM-deficient *C. trachomatis* the previously described chlamydial-dependent centrosome repositioning was significantly less evident and the inclusions displayed an altered morphology that was more susceptible to disruption of host cell
microtubules.

Conclusions
IncM helps to disrupt centrosome positioning during host cell infection by *C. trachomatis* and modulates inclusion morphology. Together, this could contribute to the observed IncM-dependent host cell multinucleation in infected cells, which results from cytokinesis inhibition. Furthermore, this ability of IncM to block host cell cytokinesis is conserved among its homologues.

*Student paper

M-39 **Discoveries from *Chlamydia trachomatis* genomics**

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Chlamydia trachomatis is an obligate intracellular bacterium that is restricted to humans. The small 1.04 Mbp genome reflects its reductive evolution and necessity for an intracellular life with dependence on host cell metabolites. Although *C. trachomatis* is the leading cause of bacterial sexually transmitted infections and preventable blindness in the world, it has been challenging to tease out the pathogenic mechanisms of disease, tissue tropism and host immune evasion to develop novel strategies for control and prevention including a vaccine. Partial and whole genome sequences ushered in a new era of discovery about *C. trachomatis* population structure and evolution shedding light on these mechanisms. While single nucleotide polymorphisms had been noted, host immune and antibiotic pressure became more evident, as did diagnostic pressure and the full extent of recombination—first recognized to involve the *omp*A gene in the early 1990s—once population genomics became possible. While recombination is rampant, specific genetic signatures for DNA insertion during recombination are not evident. Genomics enabled phylogenic studies of *C. trachomatis* that resolved strains into four distinct disease clades based on the two biological variants of the organism—invasive lymphogranuloma venereum (LGV) strains and non-invasive strains. These latter are further segregated into urogenitorectal prevalent, urogenitorectal non-prevalent and ocular strain clades. Other discoveries include a unique nine-member polymorphic membrane (*pmp*) gene family and the plasticity zone (PZ)—a region with the greatest variation among strains—that contains toxin genes and a partial tryptophan operon. Pmps are involved in host immune responses, adhesion and recombination. The lack of toxin genes in LGV strains facilitates their invasion of tissue as well as lymphatic travel to distant sites while non-invasive strains are constrained to the mucosa due to their cytotoxicity. Ocular strains have mutations in the tryptophan operon that disable tryptophan synthase production, while rectal strains can utilize indole to produce tryptophan, an essential amino acid for chlamydial replication. Although many non-rectal urogenital strains are able to use indole, they have many more mutations than previously recognized that are energetically beneficial and have disabled or impaired operon function. In summary, population genomics uncovered a rich array of genes, operons and evolutionary strategies that help explain disease pathogenesis, tissue tropism and host immune evasion, allowing this pathogen to successfully set up shop in humans. However, we still lack sufficient knowledge to develop an effective vaccine to prevent a chlamydial sweep into future generations.

M-40 **Role of *Chlamydia*-related bacteria (CRB) in human disease**

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Chlamydia-related bacteria (CRB) are frequently detected in the environment (soil, water), amoebae and various animal species, including mammals, reptiles, arthropods, isopods and fish. They share intracellular lifestyle, biphasic developmental cycle and a large core gene set (the "Pan-Genome of the Chlamydiae") with the genus *Chlamydia*. The CRB include several family-level lineages carrying a potential zoonotic threat. For example, *Parachlamydiaceae, Waddliaceae and Simkaniaceae* include species that are suggested to have human pathogenic potential, but their definite role as human pathogens waits for confirmation.

The first CRB described was *Simkania negevensis,* and an association between *Simkania* and acute bronchiolitis was shown in children from Israel. Later, some studies have presented evidence of an acute infection with *Simkania* in cases of respiratory diseases, eg. pneumonia in children and young adults. Also, *Rhabdochlamydia* spp. are found in association with respiratory infections.
Currently, *Parachlamydia acanthamoebae* and *Waddlia chondrophila* may be the most studied representatives of CRB in humans. Seroepidemiological and molecular studies as well as an establishment of an experimental pneumonia model suggest that *Parachlamydia acanthamoebae* is able to cause pneumonia. *Waddlia chondrophila* antibody is detected in association with tubal factor infertility, adverse pregnancy outcome, and lower respiratory tract infection. In addition, DNA of various CRB has been detected in human skin specimens with and without any pathology. Nevertheless, the true clinical relevance of these findings has remained controversial mainly due to the variability of the diagnostic tools used in most early studies.

In general, culture-independent techniques have greatly assisted studying the role of infectious agents in diseases of unknown etiology. Also, the classical Koch´s postulated have been revisited. The studies on the pathogenesis of CRB in humans are still hampered by the lack and/or availability of established diagnosis methods.

S-41* zDB: bacterial comparative genomics made easy

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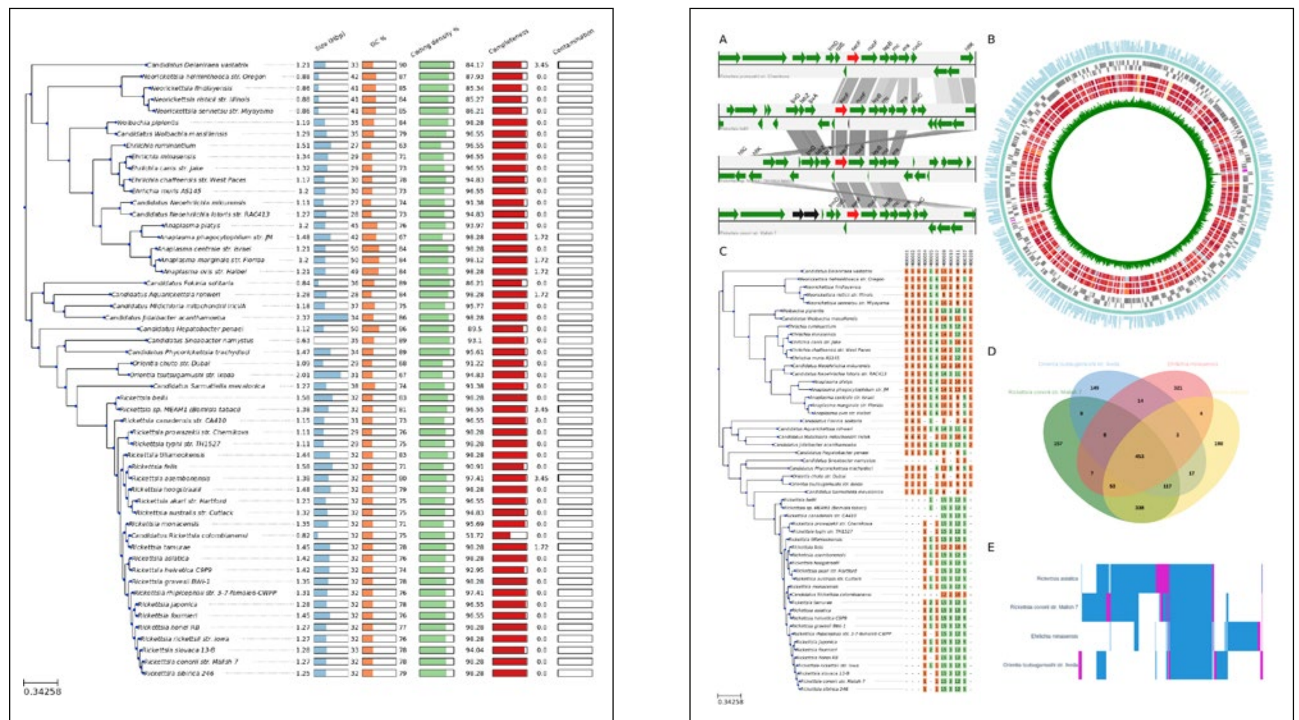
Background
The analysis and comparison of genomes relies on many specialized tools for tasks such as orthology inference, gene calling or annotation. Moreover, data integration to examine and visualize the results require significant efforts before allowing to answer scientific questions. To fill this gap, we developed zDB, an application that integrates the results of functional annotations, orthology predictions and phylogenies into an interactive web application.

Method
Starting from annotated Genbank files, zDB infers orthologs and generates a phylogeny for each orthogroup. A species phylogeny is inferred from shared single-copy orthologs. The results can be enriched with PFAM protein domain prediction, COG and KEGG annotations and SwissProt homologs. All results are stored in a database and can be accessed programmatically or visualized in a web application. The analysis and database creation are automated to limit hands-on time. We used zDB to generate a database of 58 annotated reference genomes of the *Rickettsiales* order as a proof-of-concept (Fig 1).

Results
Analyzing the 58 genomes and generating a database took 12 hours to complete on a desktop machine. The resulting web application allows to search for a specific gene or annotation, to run BLAST queries, to compare genomic regions (Fig. 2a) and whole genomes (Fig. 2b). The metabolic capacities of organisms can be compared at either the module or the pathway level (Fig. 2c). Finally, users can run queries on gene or annotation conservation across a chosen subset of genomes and display the results as a list of genes, Venn diagram (Fig. 2d) or heatmaps (Fig. 2e).

Conclusions
The features available in the web interface will make zDB useful for seasoned bioinformaticians and wet lab researchers. The *Rickettsiales* use-case demonstrates that zDB is perfectly suited to process datasets with tens of genomes on a desktop machine.



*Student paper

S-42 Exploring the genomic diversity of circulating Chlamydia trachomatis lymphogranuloma venereum strains: 2011-2017

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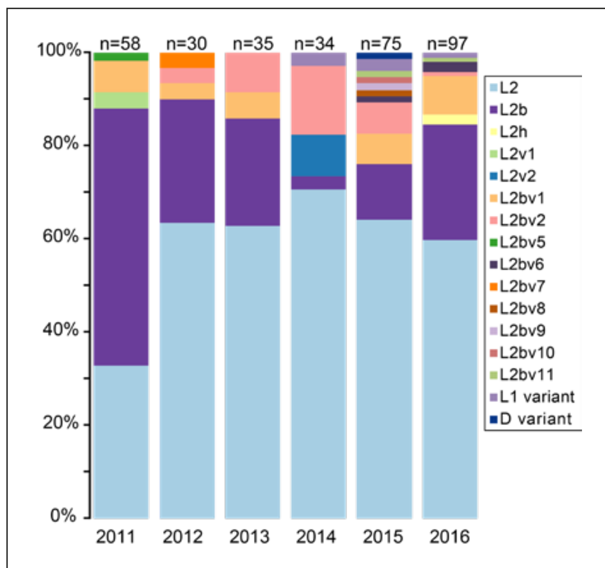
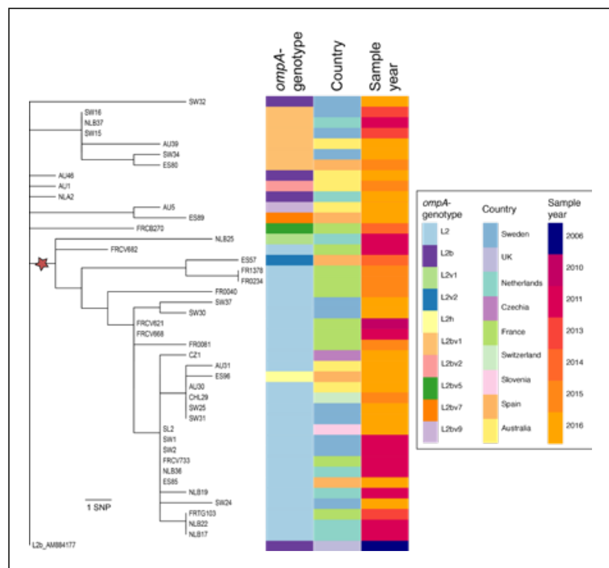
Background
Lymphogranuloma venereum (LGV), an invasive sexually transmissible infection (STI), is caused by strains from the *Chlamydia trachomatis* LGV biovar. Over the last 20 years, *ompA*-genotype L2b has replaced *ompA*-genotype L2 as the dominant LGV diagnosed globally. We investigated the diversity in LGV samples across an international collection over seven years using typing and genome sequencing.

Method
LGV-positive samples (n=321) collected from eight countries from 2011-2017 (Spain n=97, Netherlands n=67, Switzerland n=64, Australia n=53, Sweden n=37, Hungary n=31, Czechia n=30, Slovenia n=10) were genotyped for *pmpH* and *ompA* variants. To elucidate the genomic diversity, whole genome sequencing (WGS) was performed from selected samples using SureSelect target enrichment.

Results
All samples were found to carry a version of the *pmpH* gene which is associated with *ompA*-genotype L2b. However, analysis of the *ompA* gene sequence showed *ompA*-genotype L2b (n=83), *ompA*-genotype L2 (n=180) and several variants of these (n=52; 12 variant types). Using target enrichment, 42 genomes were obtained, covering diverse *ompA*-genotypes and countries. Phylogenetic analysis revealed that these *ompA*-genotypes derive from an *ompA*-genotype L2b ancestor, differing by up to eight single nucleotide polymorphisms (SNPs) per isolate. SNPs within *ompA* are overrepresented, each of which results in an amino acid change in the variable domains of OmpA (major outer membrane protein, MOMP).

Conclusions
Our data suggest that the majority of circulating isolates possess an L2b genomic backbone, within which the *ompA* gene has reverted to L2 through a single SNP mutation. This variant presents a challenge for typing. The wide diversity of *ompA*-genotypes found in these recent LGV samples suggests that *ompA* is under immunological selection. The *ompA*-genotype L2b genomic backbone may possess a selective advantage, as it continues to evolve, particularly in men who have sex with men (MSM) populations.





M-41 New insights into how *Chlamydia* effectors modulate host cell functions

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Chlamydia trachomatis (*Ct*) is a leading cause of bacteria sexually transmitted infections, yet how these obligate intracellular bacteria create a unique membrane-bound replicative compartment, the inclusion, is incompletely understood. Upon entry, *Ct* secrete a unique class of hairpin-like effectors (Incs) via the Type III secretion system. Once secreted, Incs are inserted into the inclusion membrane where they are ideally positioned at the host-pathogen interface to modulate the function of host proteins. We describe a multi-functional effector, IncE, that operates as a compact scaffold to simultaneously reprogram two unrelated host trafficking processes by employing two Short Linear Motifs (SLiMs), a strategy shared with viruses. One SLiM is located within the C-terminus of IncE and mimics a sorting motif necessary for retrograde trafficking of cargo that is recognized by Sorting Nexins (SNX)5 and SNX6, members of the Endosomal SNX-BAR sorting complex (ESCPE-1). Direct binding of IncE to SNX5/6 recruits the ESCPE-1 complex to the inclusion membrane and disrupts SNX5/6 recognition and sorting of cargo. Depletion of SNX5/6 results in enhanced production of infectious progeny, suggesting that the ESCPE-1 complex functions as a restriction factor for *Ct* infection. A second, non-overlapping IncE SLiM encodes a 6 amino motif that mimics the zero layer motif of the VAMP3 family of R-SNARES to specifically recruit Syntaxin-7 and Syntaxin-12 containing vesicles to the inclusion. Using siRNA depletion and a non-polar IncE mutant, we demonstrate that IncE-mediated recruitment of Syntaxin-7/12 vesicles subserve distinct functions for the pathogenesis of *Ct* infections: Syntaxin-7 is required for intracellular development while Syntaxin-12 is required for efficient inclusion fusion. We hypothesize that the ability of IncE to simultaneously recruit SNX5/6 and Syntaxin-7/12 vesicles to the inclusion functions synergistically to promote the *Ct* intracellular developmental cycle and that the employment of SLiMs may be a recurring motif in Inc cell biology.

M-42 **The role of type III secreted proteins during the *Chlamydia trachomatis* infectious cycle**

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Chlamydia trachomatis uses a type III secretion (T3S) system to deliver > 70 effector proteins into host cells. In general, these effectors enable host cell invasion and subsequent completion of the chlamydial infectious cycle within the intracellular vacuole (known as inclusion) where chlamydiae exclusively multiplies. Using *Yersinia enterocolitica* as heterologous host, we previously identified several candidate *C. trachomatis* T3S substrates. One of these candidates (CteG, 656 amino acid residues) was found to be transported by *C. trachomatis* into the host cell cytoplasm, where it localizes at the Golgi complex and plasma membrane. To understand the conservation of *cteG* among *Chlamydiaceae*, a reciprocal BLAST followed by a phylogenetic analysis identified 21 putative CteG orthologs in 13 *Chlamydia* species. In most cases, the sequence identity is limited to specific regions of CteG and some species harbour more than one copy of the gene. Many of these CteG orthologues are also type III secreted by *Yersinia* and can be delivered into infected host cells by *C. trachomatis*. To analyse the determinants for subcellular localization of CteG, we performed site-directed mutagenesis of *cteG* and analysed the localization of the protein after transfection of mammalian cells with plasmids encoding mutant proteins. This revealed a putative amphipathic alpha-helix in the first 20 amino acid residues of CteG that is essential for targeting a fusion between EGFP and the first 100 residues of CteG (EGFP-CteG100) to the Golgi. The same amino acid replacements had only a minor, but significant, impact in the localization of full-length CteG when ectopically expressed or delivered by *C. trachomatis* into host cells. Finally, to analyse the function of CteG, we characterized a *C. trachomatis cteG::aadA* mutant strain. This showed that CteG mediates *C. trachomatis* lytic exit, an essential step of the chlamydial infectious cycle.

S-43 Chlamydia trachomatis secretes effectors which localize to the mitochondria and induces changes in mitochondrial function

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Background
Mitochondria are critical organelles that perform a wide variety of functions including energy production and immune regulation. The majority of mitochondrial proteins are translocated to the mitochondria using distinct mitochondria targeting sequences (MTS). It has been shown that bacterial proteins can also contain MTS and localize to the mitochondria. For *Chlamydia trachomatis,* interaction with host cell organelles provides essential nutrients, promotes intracellular replication and aids in maintaining inclusion stability. However, the extent and mechanisms through which *Chlamydia* interact directly with mitochondria remain unclear.

Method
We used bioinformatics to investigate the presence of MTS in the *C. trachomatis* genome and ectopically expressed candidate genes in HeLa cells. For genes that localized with mitochondria, Type 3 secretion was evaluated using a paralogous *Yersinia pestis* secretion model. Additionally, fluorescence-activated mitochondria sorting was utilized to isolate mitochondria from infected and uninfected cells. Mitochondrial proteomes were identified by mass spectrometry.

Results
We identified 30 genes with greater than 70% probability of mitochondrial localization and ectopically demonstrated that 5 effectors were translocated to the mitochondria. Mass spectrometry revealed that two of these proteins localize to the mitochondria in a native infection. Both are putative inclusion membrane proteins shown to be Type 3 secreted. Comparison of mitochondria from infected and uninfected cells suggests that chlamydial infection affects mitochondrial protein composition. Around 125 host proteins were significantly decreased or absent in infected cells including those related to mitochondrial fission/fusion dynamics and pro-apoptotic factors. Conversely, 82 host proteins were increased in infected cells, many of which act as anti-apoptotic factors and upregulators of oxidative phosphorylation.

Conclusions
These data support that *C. trachomatis* specifically targets host mitochondria to manipulate cell fate decisions and metabolic function. This and future work will be used to expand our knowledge of chlamydial pathogenesis and elucidate mechanisms for host cell modulation that can be applied to other pathogens.

S-44 ***Orientia tsutsugamushi* effector Ank5 modulates the MHC-I pathway by targeting the transcriptional activator, NLRC5, for proteasomal degradation**

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Background

Orientia tsutsugamushi is an obligate intracellular bacterium and etiologic agent of scrub typhus, a potentially fatal disease with emerging global implications. Mechanisms enabling *O. tsutsugamushi* pathobiology and immune response regulation are poorly understood. Adaptive immunity to intracellular pathogens relies on surface exposed MHC-I molecules to recognize infected cells. We previously reported that *O. tsutsugamushi* modulates the MHC-I pathway through proteasome-dependent reduction of the MHC-I gene transactivator, NLRC5. *O. tsutsugamushi* encodes numerous T1SS Ank effectors that harbor eukaryotic-like ankyrin repeats (ARs). Most *O. tsutsugamushi* Anks also contain a C-terminal F-box domain capable of co-opting host SCF ubiquitin ligase machinery to target proteins for proteasomal degradation.

Method

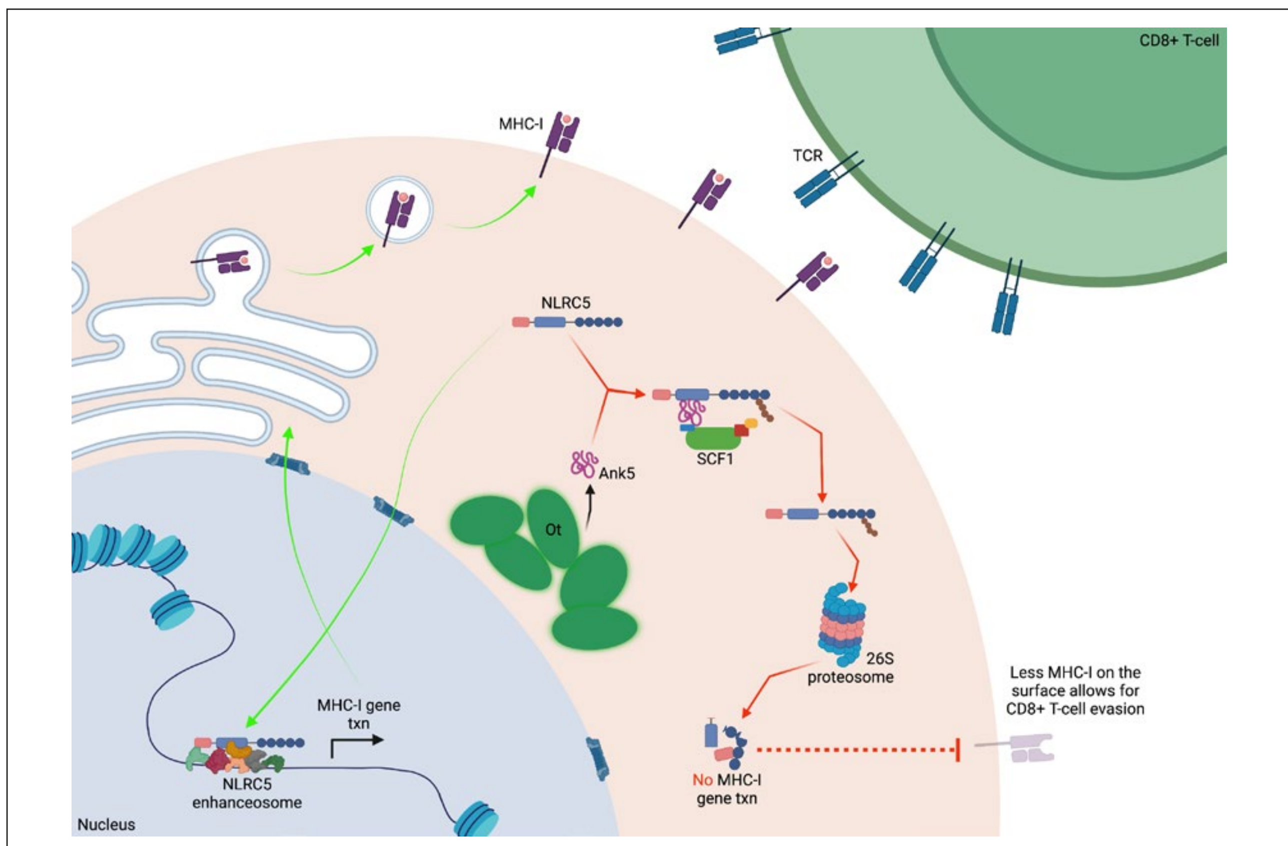
Yeast two-hybrid and co-immunoprecipitation assays were used to study protein-protein interactions. Transfected populations were often isolated via fluorescence-activated cell sorting. Flow cytometry measured surface levels of MHC-I. To validate our model during infection, we used a competitive antagonist approach to circumvent *O. tsutsugamushi* genetic intractability.

Results

Yeast two-hybrid identified NLRC5 as an Ank5 binding partner and co-immunoprecipitation of Flag-tagged Ank5 confirmed its interaction with both endogenous and over-expressed NLRC5. Further co-immunoprecipitation assays using mutant Ank5 proteins pinpointed that the interaction is dependent on AR4. Ectopically expressed Ank5 phenocopied infection-induced NLRC5 degradation in a manner that was proteasome-, F-box-, and SCF complex-dependent. Ank5 bearing a functionally inactivated F-box incapable of nucleating the SCF complex (Ank5-F-boxAAAAA), was unable to degrade NLRC5, yet prevented MHC-I gene transcription and yielded low levels of surface MHC-I. Thus, Ank5 reduces cellular levels of MHC-I by two distinct but synergistic mechanisms: it retains NLRC5 and promotes its proteasomal degradation. Host cells expressing GFP or GFP-Ank5-FboxAAAAA were infected followed by measurement of NLRC5 levels. GFP-Ank5-F-boxAAAAA functioned as a dominant negative competitor of bacterial-derived Ank5 and prevented NLRC5 degradation.

Conclusions

These data confirm that Ank5 is responsible for binding and degrading NLRC5 during *O. tsutsugamushi* infection.



M-43 **Genetic dissection of chlamydial infection biology**

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Genetic manipulation of *C. trachomatis* is now well established and multiple techniques are rapidly leading to a more thorough understanding of infection biology. Current state-of-the-art in *Chlamydia* genetics will be briefly discussed along with a consideration of gaps in the molecular toolbox. Successes in ectopic expression, gene disruption and gene deletion will be highlighted along with the emerging promise of random, transposon-based mutagenesis. Our work investigating roles of type III secretion effectors has led to an appreciation of parameters that have broad implications for chlamydial genetics. Reliable complementation or mutants as well as systems supporting mutagenesis in non-LGV chlamydiae represent two critical areas. Data will presented to emphasize the benefits of complementation and expression of engineered gene products by direct manipulation of the chromosome. Finally, we have created a new method enabling gene deletion in other *Chlamydia* including non-LGV *C. trachomatis* and *C. muridarum*. Besides opening doors to manipulation of *Chlamydia* beyond serovar L2, advantages of this approach include increased efficiency in i) generating deletion constructs in *E. coli* and in ii) chlamydial transformations. Although allelic exchange is accomplished via fluorescence reporting, curing of the endogenous chlamydial plasmid does not occur. This approach will be described and data presented showing how gene deletion in other chlamydial species is yielding sometimes surprising results.

M-44 **Regulation of *Chlamydia* gene expression by small RNAs**

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Background
Studies on *Chlamydia* gene regulation have mainly focused on transcription, but protein expression can also be controlled by small RNAs (sRNAs), which are non-coding transcripts that regulate message stability and translation initiation.

Methods
1) *Genetic screen:* Inducible overexpression of individual sRNAs in *C. trachomatis* and screen for decreased production of infectious progeny
2) *Bioinformatic and mutational analysis* to determine the target recognition sequence of a chlamydial sRNA
3) *MS2 aptamer affinity purification/RNA sequencing* to isolate mRNAs that physically interact with the sRNA
4) *Functional testing* of putative mRNA targets with translational fusion assays in *E. coli* and *C. trachomatis*

Results
We identified four chlamydial sRNAs whose overexpression decreased progeny production by more than 10-fold, including two uncharacterized sRNAs. Further analysis revealed that overexpression of the sRNA CtrR3 prevented conversion from replicating (RB) to infectious (EB) chlamydial forms, whereas CtrR7 overexpression blocked RB replication. We demonstrated that a C-rich sequence in the main predicted loop of CtrR3 is its target recognition sequence that is necessary for its overexpression phenotype. Using a combined approach of affinity purification, bioinformatic prioritization and functional testing, we showed that CtrR3 targets include YtgB, an ATPase involved in iron transport, and CTL0389, as uncharacterized inclusion membrane protein.

Conclusions
This study describes a genetic approach to investigate the functions and mRNA targets of chlamydial sRNAs, which have been characterized until now with a heterologous *E. coli* system. Our results implicate individual sRNAs in control of specific steps in the chlamydial developmental cycle such as RB replication and RB-to-EB conversion. We also describe how this inducible expression system can be used to identify the target recognition sequence and mRNA targets of a sRNA. Our findings indicate that sRNAs are likely to play an important role in the intracellular *Chlamydia* infection by coordinating the expression of specific target proteins.

S-45* Novel types of M1- and K6-specific deubiquitinases in the Chlamydia-like bacterium Simkania negevensis

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Background

Besides the regulation of many cellular pathways, ubiquitination is important for the defence against invading pathogens. Some intracellular bacteria have evolved deubiquitinase (DUB) effector proteins, which interfere with the host ubiquitin system and help the pathogen to evade xenophagy and lysosomal degradation. Most intracellular bacteria encode one or two DUBs, which mainly belong to the CE clan and typically cleave the K63-linked chains attached to bacteria or bacteria-containing vacuoles. By contrast, the respiratory pathogen *Legionella pneumophila* possesses a much larger number of DUB effectors, among them an enzyme belonging to the OTU family with a domain showing K6-specific activity and a M1-specific DUB uniquely found in this bacterium.

Method

To elucidate whether other bacteria have evolved DUBs from other enzyme classes or with certain specificities, we used bioinformatical methods to predict new DUB candidates. We then purified these candidates and tested their protease activity towards ubiquitin and other ubiquitin-like modifier.

Results

Here, we report the characterization of seven *Simkania*-encoded DUBs belonging to five different DUB classes with completely different activities. We also provide a structural basis for the M1-specificity of a *Simkania* DUB, which most likely evolved from a eukaryotic otubain-like precursor.

Conclusions

The opportunistic pathogen *Simkania negevensis* encodes a similarly high number of DUBs like the unrelated *Legionella*. Moreover, both pathogens have evolved M1- and K6-specific enzymes, which might be due to a similar life style. Interestingly, these M1- and K6-specific DUBs of *Legionella* and *Simkania* are unrelated, suggesting that their acquisition happened independently. Among all pathogens the *Simkania* DUBs are highly diverse and include DUB classes never before seen in bacteria.

*Student paper

S-46 Mechanism of cyclic-B-glucan export by ABC transporter Cgt of Brucella

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Background
Extracellular polysaccharides play critical roles in the biology of bacteria. Cyclic-ß-glucans (CßGs) are ringshaped polysaccharides critical for host-microbe interaction of *Rhizobiales*, ranging from plant symbionts to human pathogens such as *Brucella*. CßGs are synthesized in the bacterial cytoplasm and exported into the periplasmic space by the cyclic glucan transporter (Cgt), a predicted type IV ABC exporter. Our knowledge about the interactions of ABC transporters with polysaccharide cargos is limited, and substrate interaction has not been visualized so far.

Method
Here we used single-particle cryo-electron microscopy (cryo-EM), as well as a number of functional assays, to elucidate the mechanism of CßGs transport by Cgt from *Brucella abortus.*

Results
We characterized multiple conformational states, including the first ABC transporter structure bound to its polysaccharide cargo. This substrate-bound structure reveals a new binding pocket at the height of the cytoplasmic leaflet, while outward-facing conformations hint to an alternative mechanism of substrate release.

Conclusions
Our work elucidates the translocation mechanism of large, heterogenous substrates and sheds new light on protein-polysaccharide interactions. The improved understanding of glucan secretion can equip us with tools to combat *Brucella* infection, as well as a variety of other pathogens that rely on polysaccharide virulence factors.

M-45 ChlamDB: A comparative genomics database of the Planctomycetes-Verrucomicrobia-Chlamydiae superphylum

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Members of the phylum Chlamydiae are highly diverse and ubiquitous obligate intracellular bacteria. Known species infect a large range of eukaryotes including mammals, birds, fishes, arthropods and free-living amoeba. ChlamDB (https://chlamdb.ch/) is a comparative genomics database containing hundreds of genomes covering the entire Chlamydiae phylum and their closest free-leaving relatives of the PVC superphylum. The database integrates comprehensive automated protein annotations from multiple reference databases including UniProt, KEGG, COG, TCDB (transporters), STRING (protein–protein interactions) and InterPro (domains and signatures). Proteins were automatically linked to scientific articles with help of PaperBlast and STRING, two databases that rely on text mining to identify proteins that are discussed in the literature. Candidate effectors of the type III secretion system were identified using multiple in-silico algorithms and based on the identification of eukaryotic-like domains. The identification of orthologs among all PVC genomes allows users to perform large-scale comparative analyses to retrieve species specific genes or conserved genes between sets of genomes, and to identify orthologs of any protein in all genomes of the database. Phylogenetic relationships of PVC proteins and their closest non-PVC homologs in UniProt, comparisons of protein domains organization and conservation of gene neighborhood can be visualized using dynamically generated graphs. As a central resource for researchers working on chlamydia, chlamydia-related bacteria, verrucomicrobia and planctomyces, ChlamDB aims to provide access to high-quality annotations and facilitate comparative genomic analyses for the research community.

M-46 **The role of '*Candidatus* Rickettsia' as human pathogens**

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Candidatus is a prokaryotic category agreed by *The International Committee on Systematic Bacteriology* for naming the provisional status in incompletely described procaryotes. It refers to genetically characterized procaryotes that are difficult to cultivate and can only be described in limited terms. In recent years, the increasing application of molecular methods to the assessment of the diversity of prokaryotic populations in nature and to the study of complex symbioses makes it likely that numerous *Candidatus* examples are being described.

Rickettsia species are obligate intracellular α-proteobacteria associated with arthropod vectors that cause mild to severe diseases in humans and animals. *Rickettsia* genus currently comprises about 40 validated species, 20 of them pathogenic. Moreover, there are at least 50 more species in the provisional taxonomic status as *Candidatus.* Due to this bacterial genus is associated with blood-feeding arthropods, numerous rickettsial species continue to be described in a wide range of invertebrates and generate new questions on their biology, ecology, epidemiology, geographical distribution and potential pathogenicity.

To date, only '*Ca.* Rickettsia tarasevichae' and '*Ca.* Rickettsia rioja' in Europe, and '*Ca*. Rickettsia kelly', '*Ca* Rickettsia indica' and '*Ca*. Rickettsia xinyangensis' outside Europe have been associated with human disease. For the rest of the species which fulfill criteria of '*Ca*. Rickettsia' spp., their pathogenicity has not been demonstrated. Nevertheless, some of them have been detected in ticks feeding on humans, and others are present in areas where human rickettsioses are prevalent. As it is known for *Rickettsia*, its association with human disease can be found many years after its discovery. Therefore, all *Rickettsia* organisms, including strains and *Candidatus*, must be considered potential human pathogens.

S-47 The relationship of *Neoehrlichia mikurensis* with B-cell lymphomas

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Background
The tick-borne intracellular bacterium *Neoehrlichia* (*N.*) *mikurensis* is the cause of the infectious disease neoehrlichiosis, which typically features fever and vascular events including thromboembolic complications. Rituximab is an anti-B-cell biologic agent used to treat malignant B-cell lymphomas. It is also a risk factor for severe neoehrlichiosis. The discovery of patients with malignant B cell lymphomas and concomitant asymptomatic *N. mikurensis* infection before start-up of rituximab treatment led us to hypothesize that *N. mikurensis* infections could be involved in the pathogenesis of B-cell lymphomas.

Method
Clinical flow cytometry of malignant B cells. Next-generation sequencing (Illumina) and sequence analysis of the variable regions of the immunoglobulin heavy chain (IGHV) of the B-cell receptor.

Results
Five adult patients with malignant B-cell lymphoma and concomitant *N. mikurensis* infection were identified. The lymphomas featured splenic engagement and were mainly of the indolent type. The lymphoma B cells exhibited a restricted *IGHV* repertoire that was dominated by gene sequences from the *IGHV-1* and *-3* families. The lymphomas from three out of the five patients responded favorably to eradication of *N. mikurensis* infection and did not require further anti-lymphoma therapy.

Conclusions
N. mikurensis infection can have a negative impact on malignant B cell lymphomas. Eradication of this infection may halt lymphoma development.

S-48 Chlamydiaceae-like bacterium in wild Magellanic penguins (*Spheniscus magellanicus*)

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Background
Background: *Chlamydia psittaci* has been shown to cause mortality of penguin in captivity, however it is unclear whether this bacterium infects penguins in their natural environment. Serological surveys have demonstrated that it is not uncommon for wild penguins to present antibodies reactive against *C. psittaci*, but attempts to isolate *C. psittaci* or detect its DNA in wild penguins have been unsuccessful.

Method
Methods: Cloacal swabs were collected from 167 apparently healthy wild Magellanic penguins (*Spheniscus magellanicus*) at breeding colonies in Chubut, Argentina. Samples were tested with Real-Time PCR (qPCR) targeting the 23S rRNA gene of *Chlamydiaceae*. All samples positive for *Chlamydiaceae* were tested with a qPCR targeting the *ompA* gene of *C. psittaci*. Sequence identification of 16S-23S rRNA region was attempted in all samples positive for *Chlamydiaceae*.

Results
Results: DNA from *Chlamydiaceae*-like bacteria was detected in 61 cloacal swabs (36.5%). Detection did not correlate to nutritional status nor to meaningful hematological abnormalities, nor were there significant differences in prevalence among breeding colonies, age groups, or year. All samples were negative for *C. psittaci*. DNA sequences for the 16S–23S rRNA region were obtained from one sample (from an adult penguin). Phylogenetic analysis showed the presence of an organism that was closely related to unidentified *Chlamydiaceae*-like bacteria previously detected from chinstrap penguins (*Pygoscelis antarcticus*) in Antarctica (evolutionary divergence = 1.5%), from seagulls in the Bering Sea and Chile (2.9%), and from seagulls in France (3.5–3.9%). These unidentified strains were also grouped with *Chlamydiifrater* spp. isolated from flamingos in France (>6%).

Conclusions
Conclusions: There is a high frequency of DNA from *Chlamydiaceae*-like bacteria in cloacal samples from apparently healthy wild Magellanic penguins in Argentina. Further studies are necessary to clarify the taxonomy and investigate the epidemiology, pathogenicity and zoonotic potential of this group of *Chlamydiaceae*-like bacteria from aquatic birds.

M-47 **Chlamydia-related bacteria**

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The Chlamydiales order includes today more than eight family-level lineages including Chlamydiaceae and a large diversity of Chlamydia-related bacteria. The latter are (i) sharing more than 80% 16S & 23 S rRNA sequence similarity with Chlamydia, (ii) are exhibiting the same developmental stages than Chlamydia, and –like Chlamydia-, they also use T3SS effectors to manipulate the host cell. Thus, to study Chlamydia-related bacteria may help to study conserved processes such as division and core virulence mechanisms. Indeed, all Chlamydiales seem to divide thank to an actin homolog, MreB, contrarily to most bacteria and plant plastids that divide by FtsZ a tubulin homologue.
However, Chlamydiales exhibit much larger genomes, likely due to increased genes acquisition and reduced genes loss. Indeed, some amoebae-resisting bacteria are not only exposed to huge amount of bacterial genes in the protest, but also often encodes a conjugative transfer system. Thus, there are significant differences that makes chlamydia-related bacteria unique and interesting to study. Thus, our group and others showed the extended ecological niches occupied by members of the Chlamydiales, that extend from amoebae to mammals, fish and ticks. We also discovered the presence of important virulence factors such as a catalase, expressed by some chlamydia-related bacteria and in Protochlamydia, we identified the presence of CRISPR.
Finally, a very good reason to study chlamydia-related bacteria is the fact that some species, such as Waddlia chondrophila, may be pathogenic likely causing abortion in bovines and being associated to miscarriage in humans.

S-49 Evasion of the antimicrobial activities of cathelicidin host defence peptides by Waddlia chondrophila

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Background

Chlamydia trachomatis (C. trachomatis) is associated with reproductive complications including infertility and miscarriage. However, other emerging Chlamydia-related organisms such as *Waddlia chondrophila (W. chondrophila)* represent a significant and emerging health threat.

The cathelicidin family of peptides, including the human cathelicidin LL-37, the ovine cathelicidin SMAP-29 and the porcine cathelicidin Protegrin-1 (PG-1) display broad spectrum antimicrobial and immunomodulatory potential. Cathelicidins have been shown to influence the inflammatory response to pathogens, although their activity against the pathogen *W.chondrophila* has not yet been established.

Method

To determine the effects of cathelicidins upon *C. trachomatis* and *W. chondrophila* infectivity, bacteria were pretreated for 1h with varying concentrations of cathelicidins prior to infection of HEp2 cells. Cells were cultured for 24h and bacteria were quantified via quantification of genomic DNA copies and enumeration of inclusions after fluorescent immunocytochemistry. To further elucidate potential mechanisms of *W. chondrophila* innate immune evasion, HEp2 cells were again infected with *W. chondrophila* and analysed for the expression of PAD enzymes via semi-quantitative RT-PCR.

Results

The human cathelicidin LL-37 demonstrated a significant inhibitory effect on *C. trachomatis* inclusion number. However exposure to each of the tested cathelicidins promoted *W. chondrophila* infectivity as assessed by increases in genomic copy and inclusion number, and this was also correlated with increased IL-8 secretion. A significant increase in PAD enzyme gene expression was also observed in cells 24h after *W. chondrophila* infection.

Conclusions

Cathelicidins have direct inhibitory effect on *C. trachomatis*, however *W. chondrophila* shows intrinsic resistance to the activity of these peptides. Furthermore, *W. chondrophila* infection results in upregulation in PADI2 and PADI4, resulting in increased levels of protein citrullination. LL-37 is susceptible to citrullination mediated by PAD enzymes, resulting in a loss of antimicrobial and immunomodulatory activity. Taken collectively, these data represent number of concerning traits of *W. chondrophila* in terms of innate immune evasion.

S-50 The release of Simkania negevensis from infected cells depends on the modulation of host cell death and lysis

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Background

Simkania negevensis are obligate intracellular bacteria related to Chlamydia and associated with respiratory diseases. *S. negevensis* develop within a Simkania-containing vacuole, alternating between the intracellular metabolically active reticulate bodies (RB), which re-differentiate into infectious elementary bodies (EB) that are released from infected cells. Bacteria reach a developmental plateau after 3 days, and, unlike Chlamydia, are capable of prolonged infections lasting up to 15 days. So far, little is known about the mechanisms that mediate the cellular release of *S. negevensis*.

Method

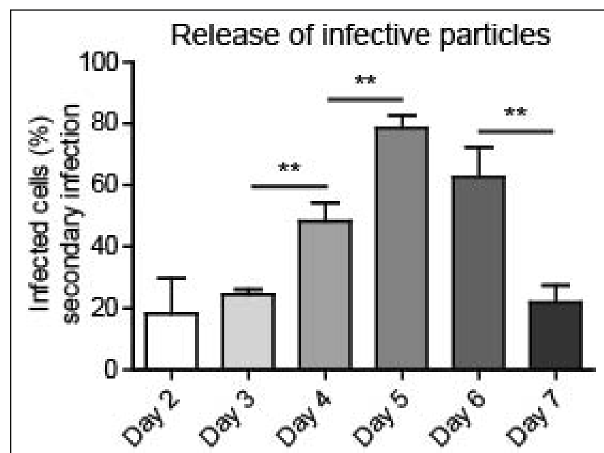
Monitoring of the secondary infection in the presence of various inhibitors was used to assess the number of infectious particles released from Simkania-infected cells. The efficacy of the apoptosis induction by TNFα and staurosporin in infected and non-infected cells was analyzed by western blot using antibodies against PARP-I. Knockout cell lines of Bax and Bak were used to study the connection between mitochondrial permeabilization and release of *Simkania* from infected cells.

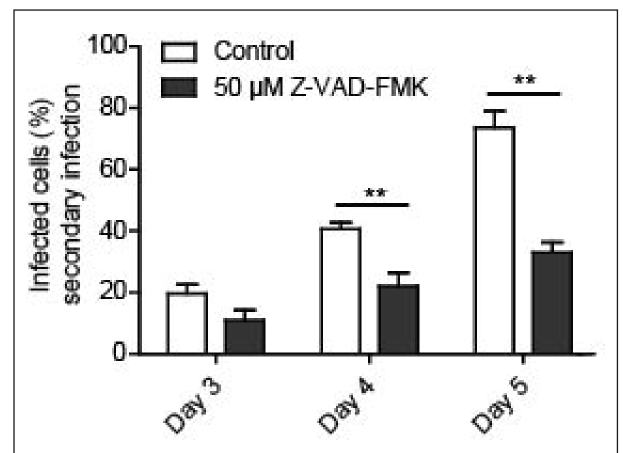
Results

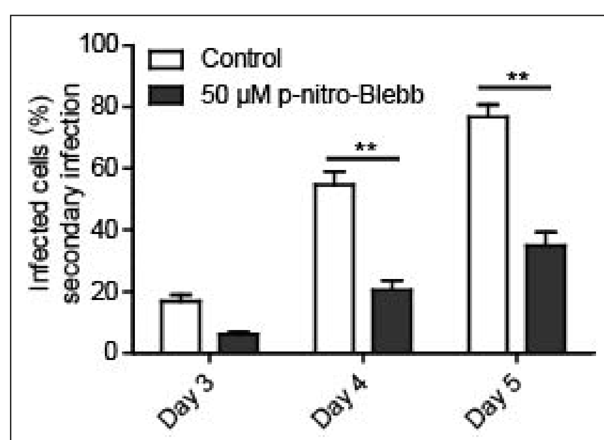
Simkania-infected epithelial HeLa and macrophage-like THP-1 cells, reduce in number during the infection. In parallel, the infectivity of the cell culture supernatant increases, starting on day 3 for HeLa and day 4 for THP-1 cells and reaching a maximum at day 5 post-infection. This correlates with the ability of *S. negevensis* to block TNFα-, but not staurosporin-induced cell death up to 3 days post-infection, after which cell death is boosted by the presence of bacteria. Mitochondrial permeabilization through Bax and Bak is not essential for host cell lysis and release of *S. negevensis*. The inhibition of caspases by Z-VAD-FMK, caspase 1 by Ac-YVAD-CMK, proteases by an inhibitor cocktail, or RIPK1 by necrostatin significantly reduces the number of released infectious particles. The inhibition of myosin II by blebbistatin also strongly affects *Simkania* release.

Conclusions

Our results point to a double mechanism of bacterial exit through host cell lysis and extrusion.







S-51* Role of Sphingolipids in Simkania negevensis Infection

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Background

Simkania negevensis (Sne) is a gram-negative bacterium of the Chlamydiales order. It displays a characteristic, obligate intracellular, biphasic life cycle, with two distinct forms. Elementary Bodies (EBs) are infectious, small, and metabolically mainly inactive, while Reticulate Bodies (RB) are replicative, bigger, and metabolically active. EBs enter the cell, where they are redifferentiating into RBs and multiply within a tubular double membrane compartment, termed Simkania Containing Vacuole (SnCV), spanning the whole cell and being in close contact to the Endoplasmic Reticulum (ER) and mitochondria.
To form the SnCV and for survival Sne depends on different host metabolites, e.g., lipids. Sphingolipids are major membrane constituents and therefore highly abundant in mammalian cells. A delicate equilibrium of different sphingolipids is crucial for the survival of the cell.

Method

To investigate the influence of changes in the sphingolipid metabolism during infection, we performed a screen of different sphingolipid metabolism inhibitors during an infection of HeLa cells to identify key players for the survival of Sne.

Results

During this screen we found first indications that treatment with Desipramine or ARC39 (Acid Sphingomyelinase (ASM) inhibitors) leads to a significant and dose-dependent reduction in the number of cells with an established infection. We could observe the same after using HPA-12, a Ceramide Transport Protein (CERT) inhibitor. CERT shuttles ceramide from the ER (*de novo* ceramide synthesis) to the Golgi apparatus (further modification). CERT inhibition was previously shown to impair normal development of *Chlamydia trachomatis* (Ctr) inclusions. In contrast after treatment with Myriocin, a potent Serine Palmitoyl Transferase (SPT) inhibitor, we could see no effect on Sne.

Conclusions

Those early findings lead to our hypothesis that the infection of HeLa cells with Sne is dependent on ASM, as well as on CERT-mediated ceramide transport, but in contrast to Ctr is not affected by the inhibition of the *de novo* synthesis pathway.

*Student paper

S-52* The occurrence of Chlamydia-related bacteria in garden birds and broiler chickens in Finland

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Background

Chlamydia-related bacteria (CRB) are ubiquitous in the environment, while some of them are also potential pathogens for animals and humans. Several species of *Chlamydiaceae* are primarily carried by birds, while the occurrence of CRB in birds has received less attention. In Australia, CRB resembling *Parachlamydiaceae*, *Simkaniaceae* and *Waddliaceae* have been detected in parrots and chickens (1,2), but the occurrence and diversity of CRB in Northern European birds is unknown.

Method

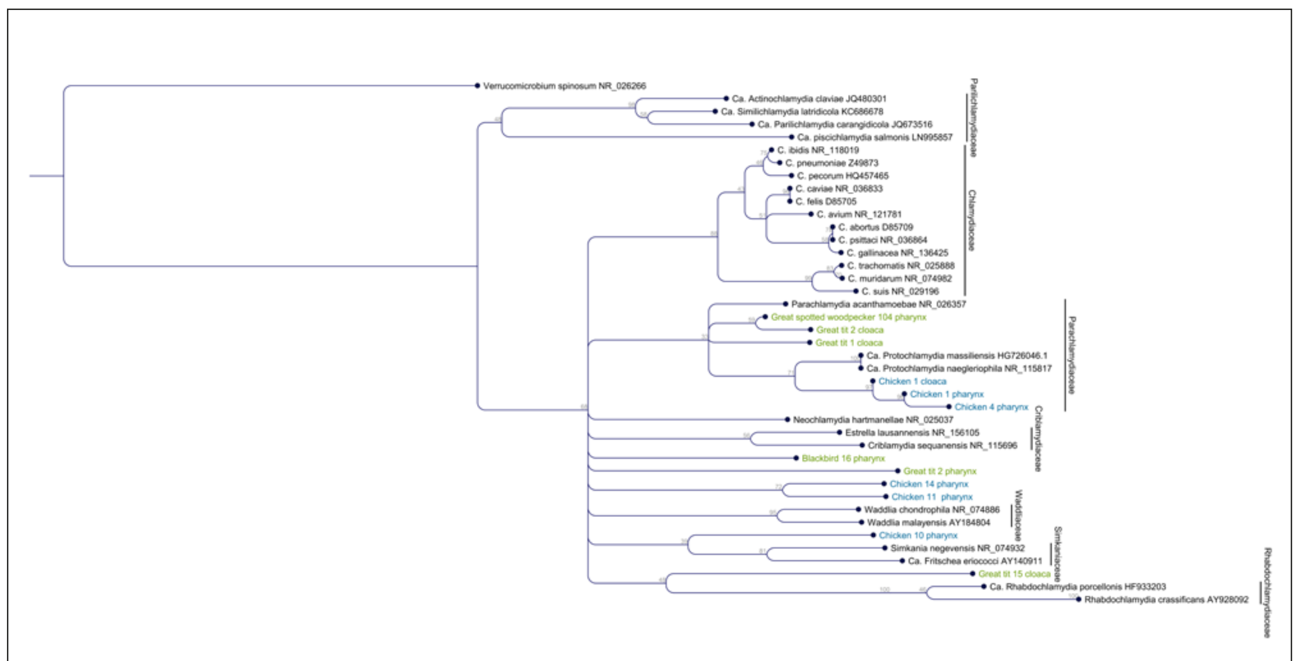
Pharyngeal and cloacal swabs were collected from 13 healthy garden birds (*Parus major*, *Turdus merula*, *Dendrocopos major*) and 20 broiler chickens in Southern Finland in 2019-2021. DNA was extracted using Zymo Microprep kit or Maxwell RSC Blood kit, and a *Chlamydiales* order-specific qPCR assay (3) was used to amplify a 210 bp fragment of the 16S rRNA gene. Amplicons were sequenced commercially and subjected to BLAST search. Maximum likelihood phylogenetic tree was constructed using CLC Bio and the neighbor joining method.

Results

13/13 garden birds (100%) and 8/20 broiler chickens (40%) tested positive in the *Chlamydiales*-specific qPCR assay in one or both sampling sites. Sequencing was successful in eight garden bird specimens and six broiler chicken specimens. Three sequences obtained from broiler chickens matched closely to *Protochlamydia naegleriophila* (>94% similarity), while nine matched to uncultured *Chlamydiales* (>93% similarity). Six of these twelve sequences grouped within the family *Parachlamydiaceae,* while four grouped outside of known families but close to each other.

Conclusions

Healthy garden birds and broiler chickens frequently carry CRB in their pharynx and cloaca in Finland. Most CRB sequences obtained from birds do not match closely to any identified species and are phylogenetically distinct from the known *Chlamydiales* families. The detection of *Parachlamydia*-like sequences in birds might indicate the presence of suitable protozoal host species. Further PCR assays and NGS methods are needed to resolve the diversity of CRB in birds.



*Student paper

M-48 **Entomology: a key tool for epidemiology of intracellular bacteria**

Idir BITAM

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Background
Infection with zoonotic intracellular bacteria can lead to several diseases in humans and animals. It is very important to develop several tools in order to better understand the development of these pathogens, thus making it possible to improve the methods of collecting arthropod vectors, diagnostic methods. the introduction of new technology which has upset the microbial world, unfortunately poor countries do not have the necessary means to carry out these diagnoses in addition to being the most affected by these infections in the world.

Method
This study is an excellent opportunity to share experience in the field and to share knowledge. We will also present the different species in the form of a directory of intracellular bacteria such as Bartonella, Chlamydia, Coxiella, Ehrlichia, Listeria, Parachlamydia, Rickettsia, as well as an inventory of the different species of arthropods and reservoirs in Africa.
Also the different techniques such as molecular biology, sequencing, NGS and Maldi-TOF are extremely effective tools allowing to have reliable and fast results and to treat infections very quickly,

Results
A mapping of arthropod species will be presented including all vector species of intracellular bacteria as well as their hosts, a directory of intracellular bacteria species will also be presented for Africa

Conclusions
The strong point of this work is the One Health concept bringing together several specialists bringing together epidemiologists, clinical microbiologists, infectiologists, entomologists, veterinarians as well as bioinformaticians, biologists, agronomists

S-53* **The North African hedgehog, a wild reservoir for ticks and fleas-borne pathogens**

Ghofrane Balti[1], **Clémence Galon**[2], **Moufida Derghal**[3], **Souheila Guerbouj**[3], **Ali Bouattour**[1], **Sara Moutailler**[2], **Youmna M'ghirbi**[4]

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Background

Wild small mammals are an important element in the emergence and the transmission of vector-borne pathogens (VBP), among which, hedgehogs known to be potential reservoir of several VBP and hosts of ticks and fleas. Surveillance of VBP in wildlife is crucial as they may be found near human and domestic animal's habitats. The aim of this study was to screen up to 48 zoonotic VBP in hedgehog's biopsies and their infesting arthropod vectors using a high-throughput microfluidic real-time PCR system.

Method

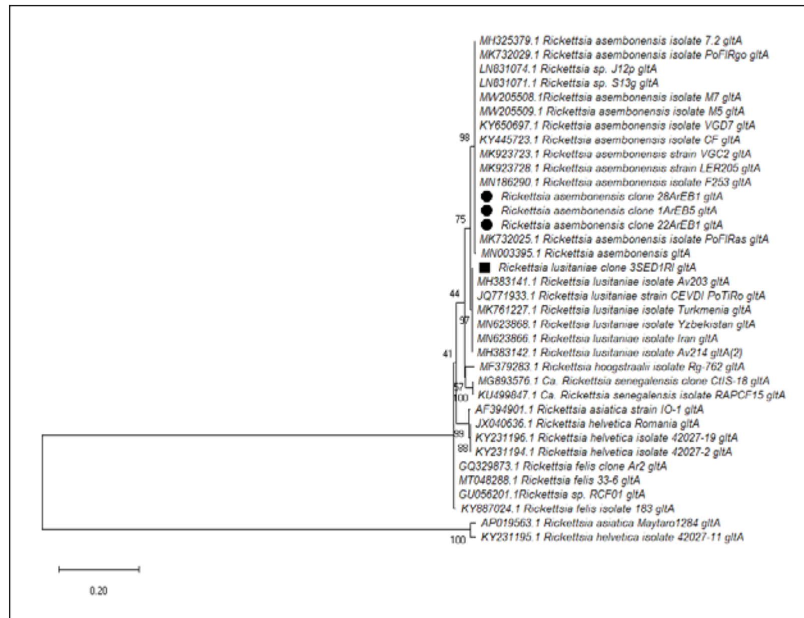
Hedgehogs were captured alive at night, in rural and sub-urban areas close to houses by inhabitants in 3 regions in Tunisia. Ticks and fleas were collected from hedgehogs. Animals were euthanized and biopsies from several organs were taken. After DNA extraction, a pre-amplification step was carried out. Microfluidic realtime PCR system screening targeting the most important vector-borne bacteria and protozoa was conducted on pre-amplified DNAs. Lastly, PCRs and Nested PCRs followed by sequencing and phylogenetic analysis were performed to confirm Microfluidic real-time PCR results.

Results

Tested hedgehogs' biopsies were reported naturally infected by *Ehrlichia ewingii*, *Candidatus Ehrlichia shimanensis*, *Coxiella burnetii* and *Rickettsia* spp. while *Haemaphysalis erinacei* and *Rhipicephalus sanguineus* ticks were infected by *Ehrlichia ewingii*, *Rickettsia* spp., *Rickettsia massiliae, Borrelia* sp., *Coxiella burnetii*, *Rickettsia lusitaniae* and *Anaplasma* sp. Moreover, *Archaeopsylla erinacei* fleas were revealed infected by *Rickettsia asembonensis, Coxiella burnetii* and *Rickettsia massiliae.* Mixed infections by two and three pathogens were detected in hedgehogs and their infesting ticks and fleas.

Conclusions

The microfluidic real-time PCR system used enabled us not only the detection of unexpected pathogens but also the co-infections in hedgehogs, ticks and fleas. These findings shed lights on the necessity of large-scale surveillance of wild reservoirs as they may contribute to the emergence and the dissemination of zoonotic VBP, which may help prevent possible human's exposure risk.



*Student paper

S-54 Seroprevalence of Q fever among blood donors and screening for Coxiella burnetii DNA in environmental dust in a French conurbation recently confronted to clustered human cases

Elsa Jourdain [1], **Xavier Lafarge** [2], **Pierre-Edouard FOURNIER** [3], **Magali Perroquin** [2], **David Abrial** [1], **Séverine Barry** [1], **Isabelle Lebert** [1], **Raquel Ceniceros** [4], **Renaud Pouget** [4], **Maxime Robert** [4], **Jaquemine Vialard** [5], **Marie Massot** [6], **Michaël Treilles** [7], **Bernard Amphoux** [3], **Lea Luciani** [3], **Elodie ROUSSET** [8]

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Background
Q fever is an air-borne zoonotic disease, due to *Coxiella burnetii*, whose main reservoir is domestic ruminants. In humans, infection is frequently asymptomatic, but may cause symptoms varying from benign flu-like syndrome to more serious and persistent debilitating troubles. Outbreaks occasionally occur, whose origin is not always elucidated. Such was the case in April-May 2017 in a French conurbation of 13 municipalities, which raised issues regarding the risk of exposure for the general population and of blood donation in this area.

Method
First, to assess the exposure of the general population, we searched for *C. burnetii* DNA in 160 dust samples collected with swabs or wipes from diverse indoor and outdoor public places, during spring 2018, using real-time and droplet digital PCR. Second, to estimate the seroprevalence of infection in blood donors, we retrospectively searched for *C. burnetii*-specific antibodies, using an immunofluorescence test in 2,500 samples donated between May and December 2017. For positive regular donors, we investigated the recent history of positivity by performing real-time PCR and serological analyses on previous donations.

Results
We detected low levels of *C. burnetii* DNA, only when using digital PCR, in 12 dust samples collected essentially from 3 municipalities, corresponding to those displaying the highest densities of small ruminants. The overall seroprevalence in blood donors was estimated at 1.96% and the proportion of seropositive results was found to vary depending of the city of residence. The serological history of the multiple donors revealed the seroconversion of 2 donors during the study period but no blood sample was positive by qPCR.

Conclusions
This study suggests that the human population residing in the considered area is moderately exposed to *C. burnetii* and provides additional evidence that the risk of transmission of *C. burnetii* via transfusion of blood
products is very low.

S-55 Prevalence and genetic characterisation of *Coxiella burnetii* in cattle in Poland

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Background
Q fever (coxiellosis) is an infectious disease of animals and humans causes by *Coxiella* (*C.*) *burnetii* and widely distributed throughout the world. Cattle and small ruminants are commonly known as a shedders of *C. burnetii*. Cattle may shed large quantities of *C. burnetii* in birth products, milk, semen, urine and feaces. The aims of this study were evaluation of seroprevalence and shedding of *C. burnetii* in cattle. Genotypes of the pathogen present in the tested specimens were also identified using MLVA and MST methods.

Method
A monitoring survey of *C. burnetii* prevalence has been performed in cattle in Poland. Sampling was conducted in different regions of Poland (16 voivodeships) in 2018-2021. In total, 2180 bovine serum samples from 801 cattle herds were tested by ELISA. Moreover, 489 specimens from 157 cattle herds such as: individual milk samples (n=407), bulk tank milk (n=58), vaginal swabs (n=20), placenta (n=3) and feces (n=1) were subjected to *C. burnetii* specific qPCR. The qPCR (IS*1111* transposon-like repetitive region) was performed using Adiavet COX RealTime PCR kit. Genotypic characterization of the strains was conducted utilizing MLVA and MST methods. MLVA was performed using 6 variable loci. MST was performed as previously described by Glazunova et al. (2005).

Results
The overall herd-level serprevalence of *C. burnetii* infection was 36.74% (801/2180). Seropositive cattle herds were recorded in all voivodeships. Shedders were detected in 29.3% (46/157) cattle herds in all tested regions. ST 61 sequence type was identified in 10 out of 18 genotyped strains. Interestingly one strain represents new sequence type. MLVA method identified three previously known genotypes: most common was J but also I and BE were recognized. Moreover, a new one genotype was detected.

Conclusions
Seroprevalence and shedding of *C. burneti*i in cattle is common and strains are genetically diverse.

S-56 Results of 12 years of the Dutch *Chlamydia trachomatis* Reference Laboratory in The Netherlands: identification of C. trachomatis plasmid free clinical isolates and other chlamydia variants

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Background
Since *C. trachomatis* variants could potentially be undetected and thus untreated, the Dutch *Chlamydia trachomatis* Reference Laboratory (DCTRL) developed in 2010 a screening programme in The Netherlands to monitor the occurrence of potential plasmid free variants and other variants in The Netherlands. In addition, the DCTRL functions as an expertise centre for questions on *C. trachomatis* strain related issues. The aim of this study is to report the finding of the DCTRL in the period 2010-2021.

Method
STD centres, Municipal Health centres, and a University centre are participating in this ongoing study and cover geographically The Netherlands. Each centre was asked to provide 25 random selected *C. trachomatis* negative samples and 10 random selected *C. trachomatis* positive samples for each quarter or specific quarters of each year expecting a 80% response rate, such that 400-500 samples per year should be collected to generate a power to detect a 2% emergence of unique strains like plasmid free variants.

Results
In the period 2010 - 2021 we collected 7763 samples (see table 1). In total we identified 8 plasmid free variants of which 6 were confirmed by an independent sample (See table 2). The Eight plasmid free variants means an incidence of 0.10%. All except one were detected in CT+ samples. As expert centre we identified the first reported case study of a female patient with bubonic lymphogranuloma venereum caused by serovariant L2b. Secondly, we identified the first person with the Swedish variant.

Conclusions
The incidence of plasmid free variants was very low, only 0.10%.. The second task of the DCTRL lead to the identification of two other variants, the first Swedish *C. trachomatis* variant in The Netherlands, and the first case worldwide of a heterosexual women with a bubonic infection with the LGV L2b strain.

Table 1: Collection of samples in the period 2010-half 2021

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
C. trachomatis +	152	138	174	199	180	180	200	194	211	232	194	160	2214
C. trachomatis -	391	331	420	499	452	439	498	474	525	575	485	460	5549
													7763

+ = *C. trachomatis* positive by NAAT, - = *C. trachomatis* negative by NAAT

Table 2: Characteristics on the identified *C. trachomatis* plasmid free variants

Year	No	Anatomical site/Gender*	Confirmation on independent sample
2010	1	VS/F	No
2011	2	P/M, A/F	yes, yes
2012	4	U/M, VS/F, O/M, U/M	yes, yes, no, yes
2020	1	U/M	yes

*VS= vaginal swab, P= proctum, A= anal, U= urine, O= oral, F= female, M= male

M-49 Functional analysis of *Bartonella* pathogenicity factors might contribute to novel therapeutic concepts

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Introduction
Bacterial adhesion to the host is the most decisive step in infections. Trimeric autotransporter adhesins (TAA) are important pathogenicity factors of Gram-negative bacteria. The prototypic TAA *Bartonella* adhesin A (BadA) from human pathogenic *Bartonella henselae* mediates bacterial adherence to endothelial cells (ECs) and extracellular matrix proteins.

Objective
To identify the exact BadA- fibronectin (FN) binding as the basis of bacterial host-cell adhesion.

Material & Methods
B. henselae strains (wild type, BadA deficient, various BadA-mutants) were exposed to FN or proteolytic FN fragments to analyze their binding affinity. Competition assays were performed using bacteria and heparin or antibodies targeting specific FN regions. The BadA-FN interaction was analyzed by mass spectrometry of cross-linked peptides. FN deficient ECs (CRISPR-CAS9) were generated and infected with various *B. henselae* strains using *in vitro* infection models.

Results
We determined the exact interaction sites between BadA and FN to be essential for host cell adhesion of *B. henselae*. BadA interactions occur mainly within the heparin-binding domains of FN. The exact binding sites were identified (i) by mass spectrometry analysis of whole-cell bacteria chemically crosslinked to FN and (ii) via expression of systematically generated *B. henselae* BadA-mutants revealing a BadA-domain-specific FN binding.

Conclusions
Interactions between TAAs (BadA) and the extracellular matrix (FN) may represent the key step for adherence of human pathogenic Gram-negative bacteria to the host. The domain-specific interaction pattern between TAAs (also expressed by, e.g., multidrug-resistant pathogens, *e.g.,* Ata of *Acinetobacter baumannii*) and FN opens the perspective to fight infections by inhibiting bacterial adhesion by a new class of antibiotics ("anti-ligands").

M-50 **Tularemia as a waterborne disease**

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Francisella tularensis is a Gram-negative, facultative intracellular bacterium causing the zoonosis tularemia. Two subspecies are responsible for human infections, subsp. *tularensis* (in Northern America) and subsp. *holarctica* (in the northern hemisphere and Australia). This pathogen can infect a wide range of animal species, including vertebrates and arthropods. Most human infections occur after direct or indirect contact with infected animals or their feces (especially lagomorphs and small rodents), consumption of food products from infected animals, or arthropod bites (mainly *Ixodidae* ticks). However, human infections related to exposure to contaminated environmental sources are increasingly reported. They represent up to 10-15% of reported tularemia cases in France.

Contamination of the hydrotelluric environment from infected animals or their carcasses is a common event. *F. tularensis* likely persists in this environment for prolonged periods. This bacterium has been detected in various types of surface water in several countries. Hypotheses to explain long-term survival of *F. tularensis* in aquatic environments include biofilm formation, interactions with protozoa such as amoebae, reduction of metabolism and replication under specific conditions (e.g., low temperature), and evolution to a viable but nonculturable (VBNC) state.

Humans can acquire tularemia through the consumption of spring water (occasionally tap water contaminated from surface water) and occupational or leisure activities in contaminated water (swimming, canyoning, fishing, etc.). The *F. tularensis* water reservoir is also involved in mosquito-borne tularemia cases observed in specific areas in Sweden and Finland. Infection of mosquitoes is considered to occur in contaminated water during their larval stage, with subsequent transmission of *F. tularensis* to the pupae and adult stages.

Tularemia mostly manifests by a regional lymphadenopathy associated with a localized infection at the *F. tularensis* inoculation site, i.e., a skin eschar (ulceroglandular form of tularemia), a conjunctivitis (oculoglandular form), or a pharyngitis (oropharyngeal form). The glandular form is a regional lymphadenopathy without detectable inoculation lesion. Inhalation of contaminated dust can lead to pneumonic tularemia and a systemic disease without apparent portal of entry of infection corresponds to the typhoidal form.

Waterborne tularemia cases primarily correspond to the oropharyngeal form. This form manifests by a pharyngitis that often persist for several weeks and resist beta-lactam therapy. Cervical lymphadenopathy develops several days following infection. The diagnosis of these forms is often difficult and the treatment delayed. In contrast, the ulceroglandular forms related to mosquito bites in Sweden and Finland are well known and readily recognized an treated by physicians.

Waterborne and mosquito-borne tularemia cases may manifest as large tularemia outbreaks. These outbreaks have been frequently reported in Turkey, and Sweden and Finland, respectively. The characterization of the aquatic cycle of *F. tularensis* is needed to fight more effectively against the emergence of tularemia. Since *F. tularensis* is a potential biological threat agent, controlling the water reservoir of this bacterium is of tremendous importance in the context of bioterrorism.

S-57 Polarized lung inflammation and Tie2/angiopoietin-mediated endothelial dysfunction during severe Orientia tsutsugamushi infection

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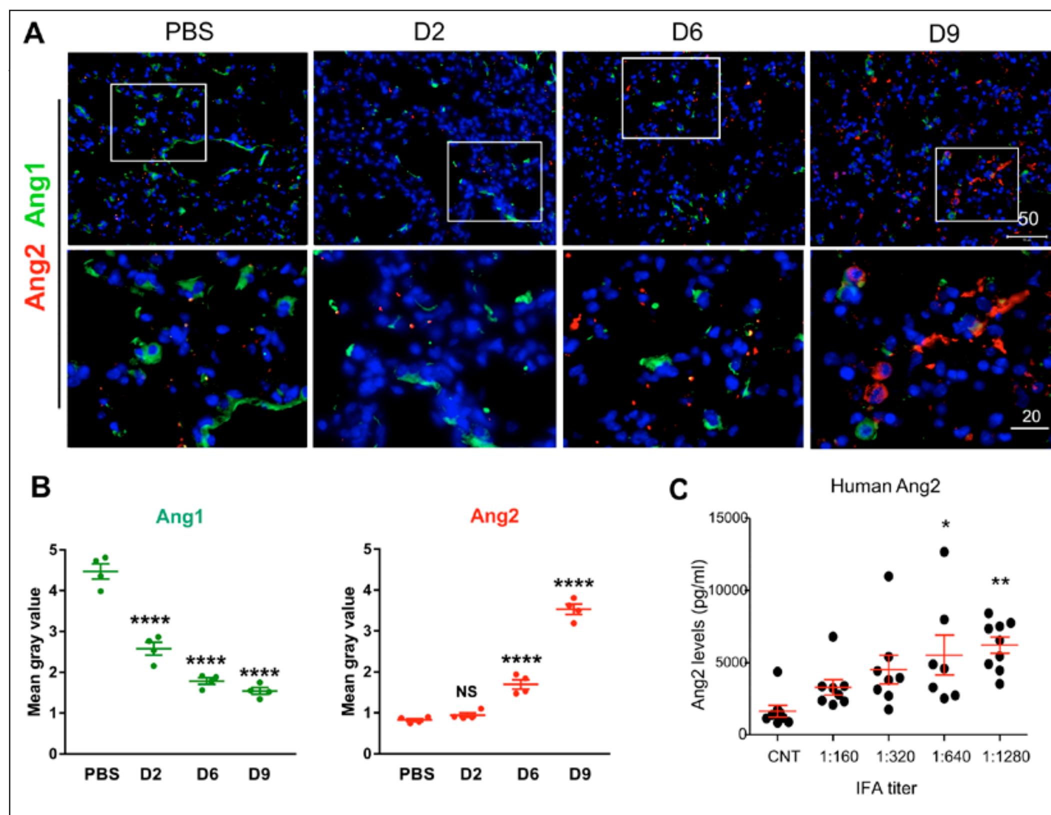
Background
Orientia tsutsugamushi infection can cause acute lung injury and high mortality in humans; however, the underlying mechanisms are unclear. Here, we tested a hypothesis that dysregulated pulmonary inflammation and Tie2-mediated endothelial malfunction contribute to lung damage.

Method
Following infection with a lethal dose of O. tsutsugamushi (i.v. route), we collected mouse lungs and spleen at 2-10 days post-infection and analyzed at the single-cell and tissue levels via multicolor FACS, IFA, qRT-PCR, and Western blot assays. We infected primary human endothelial cells (EC) and mouse macrophage (M0, M1, M2) subsets. To support our conclusion, we measured serum Ang2 and Orientia-specific IgG levels in scrub typhus patients via ELISA.

Results
Using a murine model of lethal O. tsutsugamushi infection, we demonstrated pathological characteristics of vascular activation and tissue damage: 1) a significant increase of ICAM-1 and angiopoietin-2 (Ang2) proteins in inflamed tissues and lung-derived endothelial cells (EC), 2) a progressive loss of endothelial quiescent and junction proteins (Ang1, VE-cadherin/CD144, and occuludin), and 3) a profound impairment of Tie2 receptor at the transcriptional and functional levels. In vitro infection of primary human EC cultures and serum Ang2 proteins in scrub typhus patients support our animal studies, implying endothelial dysfunction in severe scrub typhus. Flow cytometric analyses of lung-recovered cells further revealed that pulmonary macrophages were polarized toward an M1-like phenotype (CD80+CD64+CD11b+Ly6G-) during the onset of disease and prior to host death, which correlated with the significant loss of CD31+CD45- ECs and M2-like (CD206+CD64+CD11b+Ly6G-) cells. In vitro studies indicated extensive bacterial replication in M2-type, but not M1-type, macrophages, implying the protective and pathogenic roles of M1-skewed responses.

Conclusions
This is the first detailed investigation of lung cellular immune responses during acute O. tsutsugamushi infection. It uncovers specific biomarkers for vascular dysfunction and M1-skewed inflammatory responses, highlighting future therapeutic research for the control of this neglected tropical disease.



S-58 Decoding the molecular basis of premature host cell death induced by a Chlamydia trachomatis mutant deficient for the secreted effector cpoS

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Background

Host cell death is an integral part of the infection cycle of most intracellular pathogens. However, when the cell death occurs prematurely, as part of the host cell-autonomous defense, it can effectively restrict pathogen growth by depriving the microbe of its replicative niche. The obligate intracellular bacterial pathogen *Chlamydia trachomatis*, a prevalent agent of ocular and sexually transmitted diseases, uses the secreted virulence factor cpoS to suppress such defensive host cell death response. Yet, the molecular nature of this defense program remains unknown.

Method

In order to address this knowledge gap, we investigated the role of known modes of regulated cell death using genetic and pharmacologic tools. Furthermore, to identify the host proteins involved in the execution of the defense response, we conducted a pooled CRISPR/Cas9 knockout screen designed to identify gene deficiencies that protect infected cells from death.

Results

While our findings suggest that apoptosis plays a minor role, neither necroptosis nor pyroptosis contributed significantly to the premature cell death induced during infection with a cpoS mutant. Interestingly, the CRISPR/Cas9 screening did not uncover an involvement of specific cell death or immunity programs, yet revealed special metabolic requirements of the cpoS mutant not seen for wild-type bacteria. Presumably, these are a consequence of the mutants' defects in manipulating host cellular membrane trafficking.

Conclusions

Overall, this work enhances our understanding of how the action of CpoS can protect *C. trachomatis* from the host cellular defenses, a knowledge that in the future could possibly allow devising new therapeutic strategies to fight chlamydial infections.

M-51 **Chlamydial Incs: the "yes, and" effectors**

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The inclusion membrane (IM) is studded with proteins termed 'Inc' proteins. Incs are unique type III effector proteins, made only by *Chlamydia*. The genes encoding Inc proteins represent ~7% of the highly reduced chlamydial genome. Thus, their maintenance within the genome suggests that, collectively, they are inherently important to chlamydial virulence and survival. Incs are easily identified bioinformatically based on their unique structural features: at least two large hydrophobic transmembrane domains flanked by termini that are exposed on the host cytosolic face of the chlamydial inclusion. Further, the various *inc* genes are temporally expressed during early, mid, and late stages of chlamydial development; the stability of individual Inc proteins within the IM, is unknown. Our and others' data revealed that altered expression of specific Incs, resulting in IM disorganization and/or loss of IM-host interactions, negatively impacted chlamydial development. IM organization likely evolves during chlamydial development to facilitate temporal acquisition of host nutrients (e.g. amino acids or lipids) from different subcellular compartments. Hence, *Chlamydia* can limit the stress on the host cell, thus protecting their developmental niche. An understudied question is: are Incs that mediate interactions during early stages of chlamydial development degraded or reorganized to facilitate the recognition of newly targeted subcellular compartments/host signaling networks? We hypothesize that there is temporal turnover of Inc proteins in the IM to maximize inclusion stability and temporal engagement of specific subcellular compartments. Using novel chlamydial genetic techniques, we have created chlamydial strains to help us understand the impact of inducible knockdown of a single *inc* versus multiple *incs* on IM organization and chlamydial growth and development. We will also use these strains to determine the half-life of individual Incs. These studies are foundational towards understanding the possible coordinated activities of Incs and the evolution of IM content over the course of chlamydial development.

M-52 **Revisiting inclusion-organelle mimicry: evidence for inducible plasticity in inclusion architecture**

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Many obligate intracellular pathogens build specialized membrane-bound compartments within eukaryotic host cells. *Chlamydiae* replicate within one such compartment termed an inclusion. Maintenance of inclusion integrity is critical for chlamydial survival and requires a carefully balanced interaction with the host intracellular environment. Morphologically, the inclusion is commonly considered to be spherical, and although inclusion-associated fibres (tubules) have been variously reported, their role during infection remains elusive. Using live confocal imaging and ultrastructural approaches, we have further analyzed this abundant, complex, and highly dynamic filamentous network that forms in a subset of infected cells. These structures are a continuum of the inclusion membrane and increase the theoretical surface of the inclusion as well as the capacity for potential interactions with host cell proteins and organelles. Intriguingly, this plasticity in inclusion architecture increases under conditions of both bacterial and host stress. Our data suggest that the inclusion and its associated dynamic tubular network can morphologically adapt in response to external stimuli. These findings will be discussed in the context of the inclusion as an organelle mimic.

S-59 Neisseria gonorrhoeae beta-lactamase alleviates amoxicillin-induced chlamydial persistence in vitro

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Background

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) cause most bacterial sexually infections worldwide. Both pathogens primarily target the endocervical and urethral epithelium, and frequently exist as co-infections. Beta-lactam exposure induces chlamydial persistence, a reversible non-infectious divergence from normal development. NG Beta-lactam resistance is commonly conferred by beta-lactamase production. We hypothesize that NG beta-lactamase can limit amoxicillin (AMX)-induced CT persistence *in vitro* and in a mouse vaginal model of CT/NG co-infection.

Method

HeLa cells in 24-well plates were infected with CT in the presence of AMX to induce chlamydial persistence. Next, CT-infected cells, in the continued presence of AMX, were exposed to NG grown in transwell inserts placed in the HeLa/CT culture medium to allow NG to release beta-lactamase without directly contacting the CT-infected host cells (preventing host cell cytotoxicity). Chlamydial development and infectious EB production were evaluated to determine prevention or reversal of chlamydial persistence. Additionally, we evaluated if beta-lactamase producing NG (PPNG) infection could be established in AMX-treated mice.

Results

In vitro, AMX-exposed CT showed the expected 1) aberrant developmental forms and 2) reduced infectious EB production characteristic of persistence. PPNG partially prevented or reversed AMX-induced chlamydial persistence, depending on time of NG addition, resulting in largely normal CT developmental forms and increased infectious EB production. Similar *in vitro* results were observed for *Chlamydia muridarum* (CM), used to model CT infection in mice. PPNG infection of AMX-treated mice, at AMX levels sufficient to induce CM persistence, resulted in vaginal shedding of live PPNG for up to 10 days.

Conclusions

PPNG beta-lactamase production is sufficient to prevent or reverse CT AMX-persistence *in vitro*. Evaluation of the potential for PPNG infection of AMX-treated CM-infected mice to alleviate chlamydial persistence is underway. Such alleviation would provide novel evidence of bacterial interaction in CM/NG *in vivo* co-infection.

POSTERS

P-01* **Intrapenile infection of boars with Chlamydia trachomatis E/Bour failed to induce a strong immune response**

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Background
The aim of the present study was to establish a swine infection model for male urogenital infections with *Chlamydia (C.) trachomatis*.

Method
Five *C. suis*-negative boars were infected with 108 *C. trachomatis* E/Bour TCID50 by means of urine catheters, while five other *C. suis*-negative boars were mock infected. Both urogenital excretion as well as serum and mucosal antibody titers were monitored by weekly sampling until euthanasia three weeks post infection (p.i.). PBMCs were isolated on day 17 p.i. for analysis of T cell proliferative responses. During necropsy, tissue samples of the urogenital tract, spleen, local draining lymph nodes and the intestinal tract were collected for detection of *C. trachomatis* by real time PCR and immunofluorescence staining.

Results
No clinical signs could be observed. Urogenital excretion of *C. trachomatis* could be confirmed, while no mucosal antibody responses were detected. Three weeks p.i., very low anti-Bour serum antibody titers were found in two of five experimentally infected animals. On day 17 p.i., weak T cell proliferative responses were observed after in vitro re-stimulation with recombinant major outer membrane protein (MOMP) of isolated PBMCs. During necropsy, macroscopic lesions of the urogenital tract were more noticeable in the infection group in comparison to the control group. Presence of *C. trachomatis* was demonstrated by real time PCR in a number of urogenital tissues from the animals in the infection group. Confirmation by immunofluorescence staining has yet to be performed, but results will be presented.

Conclusions
In conclusion, additional research will be necessary to improve and/or fine tune the *C. trachomatis* experimental infection model in boars before it can be used for further studies on *Chlamydia*-host interactions.

*Student paper

P-02* **Systematic identification of effector-effector interactions in chlamydia trachomatis**

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Background
During pathogenesis, *Chlamydia trachomatis* relies on an arsenal of proteins termed "effectors" which are translocated into the host cell to manipulate host cell processes. These effectors act in concert during infection, but to the detriment of understanding pathogenesis, they are often studied individually. Pathogens must strike a balance between manipulating the host for its replication, abrogating host defenses and preventing premature host death. Some pathogenic bacteria finetune effector activity using effectors that antagonize or synergize with each other. With such a complex intracellular lifestyle, we hypothesized that *C. trachomatis* uses similar strategies to finetune pathogenesis. To explore this, we screened for antagonizing or synergizing activity between *C. trachomatis* effectors.

Method
We used budding yeast, *Saccharomyces cerevisiae,* as a proxy for host cells in which pair-wise combinations of *C. trachomatis* effectors were expressed. Because bacterial effectors often target highly conserved cellular processes that extend from mammals to yeast, the overexpression of these effectors can result in observable yeast growth inhibition. These differences in growth were leveraged to screen for functional interactions between 246 *C. trachomatis* effectors in over 60,000 pair-wise combinations to identify functional synergy or antagonism between effectors.

Results
We identified 15 antagonistic and 2 synergistic functional interactions between 26 *C. trachomatis* effectors. Using the Yeast-Two-Hybrid protein-protein interaction assay, we found that at least 5 of these pairs physically interact, a hallmark of direct effector regulators known as metaeffectors.

Conclusions
We are now working towards the molecular characterization of these functional and physical effector-effector interactions through *in vitro* study and understanding their role in pathogenesis through infection-based experiments. Altogether, this work represents the first comprehensive examination as to how the *C. trachomatis* effector arsenal works together during infection. It is already revealing new effector biology that will complement the large area of study around individual effectors during pathogenesis.

*Student paper

P-03* The type III secretion effector CteG mediates host cell lytic exit of Chlamydia trachomatis

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Background
We previously identified the *Chlamydia trachomatis* type III secretion effector CteG and showed it localizes at the host cell plasma membrane at late stages of the infectious cycle of this obligate intracellular pathogen. Therefore, we envisioned a role of CteG in chlamydial egress from host cells, which can occur by extrusion of the vacuole where *C. trachomatis* resides intracellularly or by host cell lysis. We also studied the relation between CteG and Pgp4, a transcription regulator encoded in the *C. trachomatis* virulence plasmid that has previously been shown to mediate chlamydial lytic exit.

Method
HeLa cells were infected with the following *C. trachomatis* strains: wild type, CteG-deficient, CteG-deficient carrying a plasmid overproducing CteG, or related strains encoding or lacking Pgp4, followed by quantification of infectious chlamydiae in the culture supernatants, of host cell cytotoxicity, and of CteG production by immunoblotting and immunofluorescence microscopy.

Results
From 48h post-infection, we found more infectious chlamydiae in the culture supernatant of cells infected by wild-type *C. trachomatis* comparing to the *cteG* mutant strain. This was CteG-dependent as it was complemented in cells infected by the CteG-deficient strain carrying plasmid-encoded CteG. Furthermore, we detected a CteGdependent defect on host cell cytotoxicity, indicating that CteG mediates chlamydial lytic exit. We showed that Pgp4 does not regulate the production or localization of CteG. However, a strain lacking CteG and Pgp4 was as defective in promoting host cell cytotoxicity as strains lacking only CteG or Pgp4. Furthermore, CteG overproduction in a plasmid suppressed the host cell cytotoxic defect of CteG- and Pgp4-deficient chlamydiae.

Conclusions
Our study unveils CteG as the first chlamydial type III secretion effector involved in host cell lytic exit. Additionally, it indicates that CteG and Pgp4 participate in a single cascade of events involving multiple layers of regulation, culminating in host cell lysis and release of chlamydiae.

*Student paper

P-04 **Manipulation of intracellular microbial sensors by the stealth pathogen Coxiella burnetii**

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Background

Coxiella burnetii is a highly infectious pathogen causing the zoonosis Q fever. *Coxiella* can invade and develop in alveolar macrophages and trophoblasts. During infection, *Coxiella* forms an intracellular replicative niche named *Coxiella*-Containing Vacuole (CCV). This is driven by effector proteins secreted by the bacterium into the host cell cytoplasm via a Type 4b Secretion System (T4SS). *Coxiella* can dampen, in a T4SS-dependent manner, the inflammatory response of infected cells to promote intracellular persistence. Analysis of our *Coxiella* mutant library led to the identification of 4 mutants displaying defects in cytoprotection of the infected cells.

Method

We focused on *icaB*::Tn mutant, as this gene encodes a hypothetical protein with features corresponding to secreted effectors. Bioinformatics analysis indicated that IcaB possesses partial structural homology with NLRs, which is unprecedented for bacterial effectors. We thus tested the localisation and interaction of IcaB with a set of NLRs using microscopic and co-immunoprecipitation techniques.

Results

We confirmed that *Coxiella* secretes IcaB in a T4SS-dependent manner. Interestingly, IcaB colocalised with NLRP1, NLRP3, NLRP5 but not with NLRP2, NLRP10 or NLRP11. Furthermore, we could identify a direct interaction between IcaB and several NLRs by co-immunoprecipitation.

Conclusions

This project opens new perspectives on how NLRs are manipulated by *Coxiella* during the course of infection.

P-05 No increased risk of mature B-cell non-Hodgkin lymphoma after Q fever detected: results from a 16-year ecological analysis of the Dutch population incorporating the 2007–2010 Q fever outbreak

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Background

A causative role of Coxiella burnetii (the causative agent of Q fever) in the pathogenesis of B-cell non-Hodgkin lymphoma (NHL) has been suggested, although supporting studies show conflicting evidence. We assessed whether this association is present by performing a detailed analysis on the risk of mature B-cell NHL after Q fever during and after the largest Q fever outbreak reported worldwide in the entire Dutch population over a 16-year period.

Method

We performed an ecological analysis. The incidence of mature B-cell NHL in the entire Dutch population from 2002 until 2017 was studied and modelled with reported acute Q fever cases as the determinant. The adjusted relative risk of NHL after acute Q fever as the primary outcome measure was calculated using a Poisson regression.

Results

Between January 2002 and December 2017, 266 050 745 person-years were observed, with 61 424 diagnosed with mature B-cell NHL. In total, 4310 persons were diagnosed with acute Q fever, with the highest incidence in 2009. The adjusted relative risk of NHL after acute Q fever was 1.02 (95% CI 0.97–1.06, P¼0.49) and 0.98 (95% CI 0.89–1.07, P¼0.60), 0.99 (95% CI 0.87–1.12, P¼0.85) and 0.98 (95% 0.88–1.08, P¼0.67) for subgroups of diffuse large B-cell lymphoma, follicular lymphoma or B-cell chronic lymphocytic leukaemia, respectively. Modelling with lag times (1–4 years) did not change interpretation.

Conclusions

We found no evidence for an association between C. burnetii and NHL after studying the risk of mature B-cell NHL after a large Q fever outbreak in Netherlands.

P-06 **Can infection with Neoehrlichia mikurensis be acquired from transfusion of blood components?**

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Background

Neoehrlichia (*N.*) *mikurensis* is an intracellular tick-borne bacterium that can cause infections characterized by fever and vascular events in humans. Asymptomatic infections have also been reported, but no transfusiontransmitted infections have been described so far. Novel findings have raised the question if blood transfusion is a possible route of transmission of this emerging pathogen, which appears to infect vascular endothelium. In view of the very low genetic variability of the *N. mikurensis* genome, new molecular tools are needed to determine if strains carried by a blood donor and a blood recipient are identical or not.

Method

Trace-back investigation to identify infected recipients of blood components. Development of Single nucleotide polymorphism (SNP)-analysis to discriminate between strains of *N. mikurensis*.

Results

Two asymptomatic blood donors who tested positive for *N. mikurensis* by real time PCR were discovered. It was suspected that transmission of *N. mikurensis* infection had occurred from one of the blood donors to one patient who also tested positive for *N. mikurensis*. A comparison of the whole genome sequences of *N. mikurensis* strains derived from 3 Swedish patients revealed 6 possible locations that could be used for SNP-analysis to discriminate between strains of *N. mikurensis*. Primers were designed against these locations and Real Time PCR was used to amplify the target sequences followed by Sanger sequencing in both directions. Amplification of 4/6 genes was successful for two clinical isolates of *N. mikurensis*. However, the low bacterial burden in healthy blood donors requires further optimisation of the amplification steps of this assay before it can be applied.

Conclusions

Although it is too early to determine if blood transfusions are a means of transmission of *N. mikurensis* infections, caution is warranted in view of multiple reports of transfusion-transmitted infections by related species within the family of *Anaplasmataceae*.

P-07* Comparison of different approaches to assess the number of Chlamydiales bacteria in a given sample

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Background

Bacterial quantification is crucial to standardize scientific experiments in research laboratories. While optical density is the gold standard used with extracellular bacteria, other methods were developed for intracellular bacteria such as *Chlamydiales.* Inclusion forming units (IFU) counting is a commonly used procedure to assess the number of *Chlamydiaceae* but it is less common when working with *Chlamydia*-related bacteria, since specific qPCR have been developed for these bacteria. Another attractive, but underused, approach to quantify *Chlamydiales*, is flow cytometry (FCM), a fast and cheap titration method that proves its worth for viruses.

Method

In this study, we quantified *Chlamydiae* in host cells by IFU counting, qPCR and FCM. We compared bacterial quantification by these different approaches during the course of an infection cycle and highlighted the pros and cons of each technique when quantifying either the extracellular and infectious form of the bacteria (Elementary bodies, EBs) or the intracellular and replicative form (Reticulate bodies, RBs). Experiments were performed on *Chlamydia trachomatis* and *Waddlia chondrophila,* which are ideal model-organisms respectively for *Chlamydiaceae* and *Chlamydia*-related bacteria.

Results

IFU counting is necessary to quantify infectious EBs. However, it is time consuming and might underestimate bacterial load. qPCR is a sensitive and fast method to quantify bacteria, however it overestimates bacterial load, since there is no possibility to distinguish between dead and live bacteria. Similarly to qPCR and for the same reason, FCM might overestimate bacterial load. However, it brings new insights into bacterial quantification by allowing the counting not only of EBs but also of infected cells at mid and late times points post-infection.

Conclusions

The three methods are pertinent to quantify *Chlamydiales*. However, users should be aware of their pros and cons and choose the method best suited to their needs.

*Student paper

P-08 A Novel Flow Cytometric Approach for the Quantification and Quality Control of Chlamydia trachomatis Preparations

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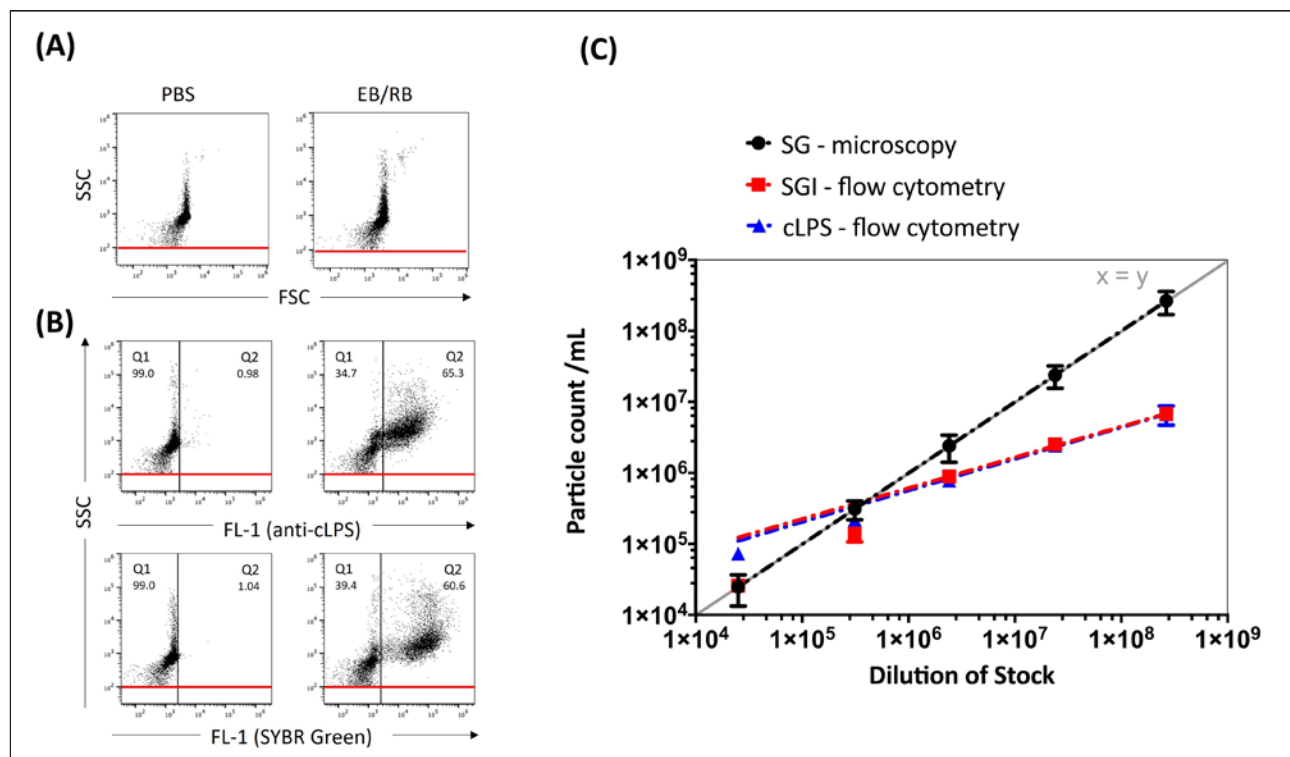
Background
Chlamydia trachomatis is an obligate intracellular pathogenic bacterium with a biphasic developmental cycle manifesting two distinct morphological forms: infectious elementary bodies (EBs) and replicative intracellular reticulate bodies (RBs). Current standard protocols for quantification of the isolates assess infectious particles by titering inclusion-forming units, using permissive cell lines, and analyzing via immunofluorescence. Enumeration of total particle counts is achieved by counting labeled EBs/RBs using a fluorescence microscope.

Method
For a better assessment of *C. trachomatis* preparations, we developed a simple and time-saving flow cytometrybased workflow for quantifying small particles, such as EBs with a size of 300 nm. This included optimization of gain and threshold settings with the addition of a neutral density filter for small-particle discrimination. The nucleic acid dye SYBR® Green I (SGI) was used together with propidium iodide and 5(6)-carboxyfluorescein diacetate to enumerate and discriminate between live and dead bacteria.

Results
We found no significant differences between the direct particle count of SGI-stained *C. trachomatis* preparations measured by microscopy or flow cytometry ($p > 0.05$). Furthermore, we completed our results by introducing a cell culture-independent viability assay. Our measurements showed very good reproducibility and comparability to the existing state-of-the-art methods.

Conclusions
In summary, the evaluation of *C. trachomatis* preparations by flow cytometry is a fast as well as reliable method and thus facilitates an improved assessment of the quality of *C. trachomatis* preparations for downstream applications.



P-09* Applying phenotypic phage display to identify antigenic targets involved in Chlamydia trachomatis infection

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Background

Monoclonal antibodies are currently utilized in multiple clinical settings, including diagnosis and treatment of diseases. One method for the development of these antibodies is through phage selection. This is a robust technique that has been widely successful in drug discovery and antibody development.

Method

In this study, phenotypic phage selection was performed on *C. trachomatis* EB material. This material is derived using differential centrifugation of manually lysed infected cells to enrich the population of EBs. Following selection, scFv antibody fragments were screened to determine potential binders using a cell based ELISA. 18 scFvs indicated binding and these were selected and reformatted into IgGs.

Results

All 18 IgGs were further screened using confocal microscopy to visualize binding patterns of infected cells, 24hpi. This screening revealed three distinct binding patterns: diffuse cellular (9), inclusion localized (5), or inclusion localized with filaments (4). IgGs which localized to the inclusion were further analyzed throughout a cycle of infection, 16hpi to 72hpi, in order to determine how target abundance changed throughout. Interestingly, many of the targets displayed diverse binding patterns during the infection cycle, indicating that there may be multiple protein targets recognized by the antibodies. A selection of IgGs were chosen for target discovery by co-immunoprecipitation of infected cell lysate followed by mass spectrometry analysis. Binding profiles from mass spectrometry revealed how each IgG pulled down distinct populations of proteins from both human and *C. trachomatis* origins, strongly indicating that each IgG binds to a different protein target involved in *C. trachomatis* infection.

Conclusions

These results suggest that utilizing the technique of phenotypic phage display is a viable and robust method to uncover proteins involved in infection. Further study of these IgGs will be performed to identify each target and characterize the role of each in infection.

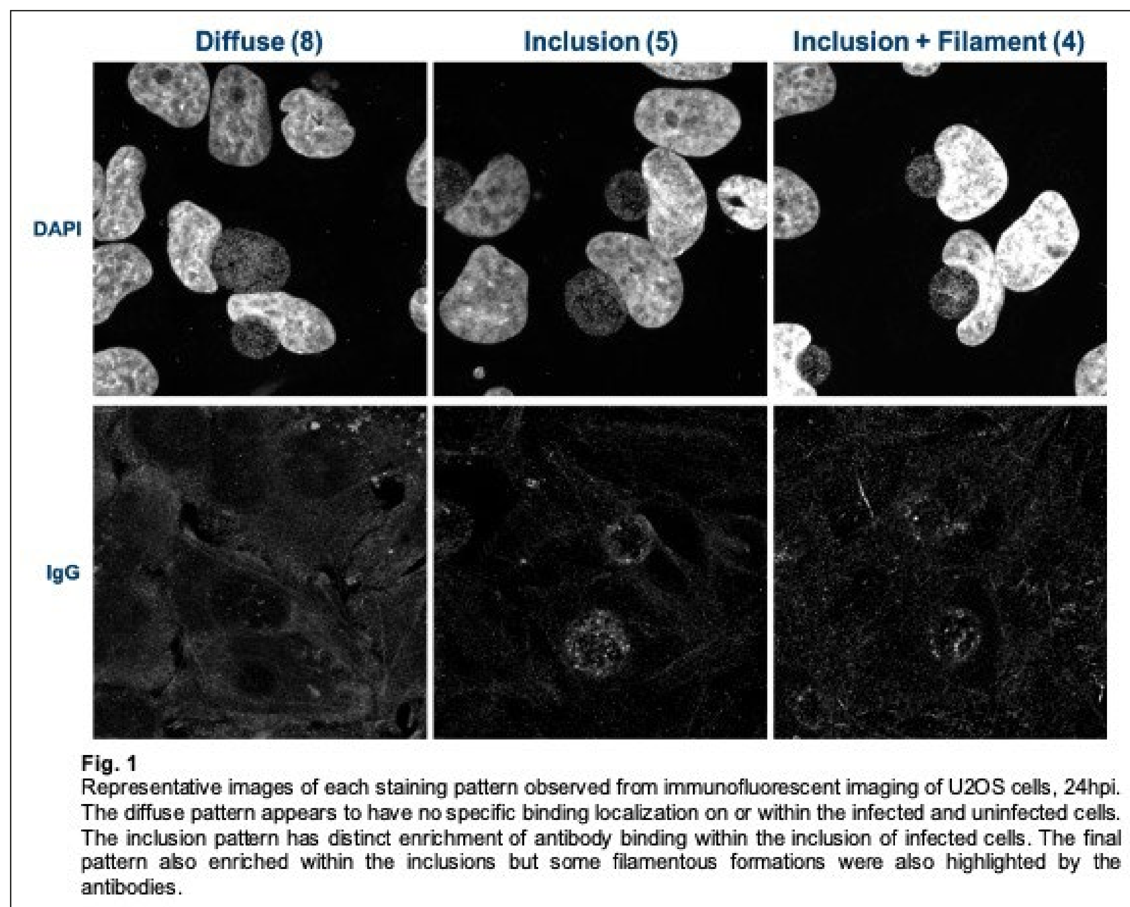

Fig. 1
Representative images of each staining pattern observed from immunofluorescent imaging of U2OS cells, 24hpi. The diffuse pattern appears to have no specific binding localization on or within the infected and uninfected cells. The inclusion pattern has distinct enrichment of antibody binding within the inclusion of infected cells. The final pattern also enriched within the inclusions but some filamentous formations were also highlighted by the antibodies.

*Student paper

P-10* **A new chlamydiales-specific t3ss effector: a key player in chlamydial persistence?**

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Background

The *Chlamydiales* order contains obligate intracellular bacteria (such as *Waddlia chondrophila* and *Chlamydia trachomatis*) sharing a common biphasic developmental cycle characterized by infectious, non-replicative elementary bodies and non-infectious, replicative reticulate bodies. When exposed to stress stimuli, reticulate bodies differentiate into enlarged bacteria called aberrant bodies, which are thought to play a role in persistence. However, very little is known about the mechanisms underlying their formation and recovery.

Method

RNA-sequencing performed on *Waddlia* aberrant bodies provided a list of genes up and downregulated upon iron starvation. We focused on candidate genes conserved in the *Chlamydiales* order. By using RT-qPCR, immunoblot and immunofluorescence we are currently studying their expression profile during the regular life cycle as well as in aberrant bodies induced by different stress stimuli. Co-immunoprecipitation is used to identify interacting proteins and elucidate the function of the selected candidates.

Results

We confirmed that *ispA (iron starvation protein A)*, a *Chlamydiales*-specific gene, is upregulated at the RNA and protein levels in aberrant bodies induced by iron starvation. Bioinformatic tools predict IspAWc and its *C. trachomatis* homolog as putative type III secretion effectors. We confirmed secretion of both using *Yersinia enterocolitica* as heterologous system. Interestingly, *ispA* clusters with two neighboring genes also very conserved among *Chlamydiales*, which suggests that they could be functionally linked. We are thus currently investigating the expression profile of these genes in *Waddlia* and *C. trachomatis*, and whether the corresponding proteins are part of the same complex.

Conclusions

Our preliminary results reveal IspA, a conserved, *Chlamydiales*-specific T3SS effector, as a marker of aberrant bodies induced by iron starvation. Future experiments are required to elucidate the role of this conserved protein in *Waddlia* and *C. trachomatis.* Furthermore, the conservation of the *ispA* locus among members of the *Chlamydiales* order suggests that the proteins encoded by this locus might physically and/or functionally interact.

*Student paper

P-11 **Autologous venous vascular reconstruction compared to non-autologous reconstruction as surgical treatment for vascular infections in vascular chronic Q fever patients**

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Background
There is no consensus on the preferred type of surgical intervention for an infected abdominal aneurysm or infected vascular prosthesis caused by *Coxiella burnetii* in chronic Q fever (CQF) patients. Autologous venous vascular reconstruction is an attractive surgical treatment option, but comparative research is lacking.

Method
We compared autologous venous vascular reconstructions with non-autologous reconstructions in a cohort of proven vascular CQF patients. Patients with infected (endo-)vascular grafts as well as primary infected abdominal aortic aneurysms were included. Primary outcome was vascular chronic Q fever-related events. Secondary outcomes were all-cause mortality, vascular chronic Q fever-related mortality, and vascular chronic Q fever recurrence.

Results
In total, 81 patients with 90 vascular reconstructions were included in the study. Autologous reconstructions were performed in 27 (30.0%) cases and non-autologous reconstructions in 63 (70.0%) cases. The median followup time was 37.5 months (Interquartile range: 9.8 – 69.0 months). The risk of early all-cause mortality, Q fever related mortality (adjusted hazard ratio (HR) both: 1.82 95% CI 0.39 – 7.84) and Q fever related events (HR 1.46 95% CI 0.33 – 5.83) were not different. The adjusted HR for late Q fever-related events was 0.08 (95% CI 0.02 – 0.43) and for vascular chronic Q fever recurrence 0.12 (95% CI 0.03 – 0.44). No significant differences were observed for the risk of late all-cause mortality (HR 0.67 95% CI 0.22 – 2.06) and late Q fever-related mortality (HR 0.14 95% CI 0.01 – 1.47).

Conclusions
Autologous venous reconstructions for vascular infections in proven CQF results in less late Q fever-related events compared to non-autologous reconstructions.

P-12 The role of cell-to-cell spread in Wolbachia vertical transmission

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Background

Vertical transmission — the inheritance of an infection from parent to offspring — is crucial for the survival of endosymbiotic bacteria and a rich source of host-microbe interactions. The intracellular insect endosymbiont *Wolbachia pipientis* is unusual in that it not only maintains an infection in the germline (the cells that become the progeny), but it also retains the ability to spread from somatic (non-germline) tissue into the germline to colonize naïve hosts. Understanding the strategy by which *Wolbachia* infects offspring is critical for pinpointing hostmicrobe interactions that ensure its survival; however, the contributions of somatic and germline *Wolbachia* to vertical transmission remain unclear.

Method

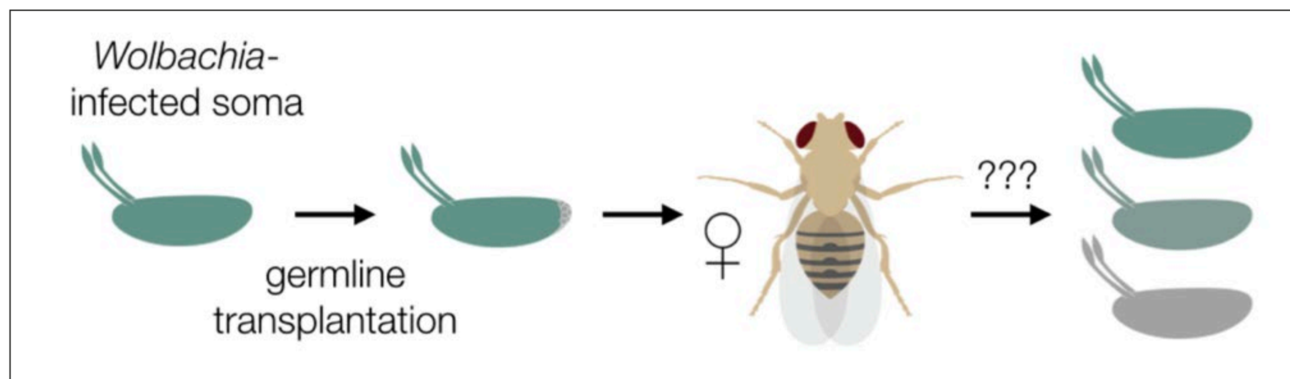
We transplanted uninfected germlines into infected *Drosophila melanogaster* individuals and, conversely, infected germlines into uninfected individuals. To assess the efficiency of vertical transmission, we quantified the bacterial titer in eggs and visualized infection in the ovaries from these mothers.

Results

We found that all mothers from both soma-infected and germline-infected transplantation experiments were capable of laying eggs with *Wolbachia* titers comparable to the stably-infected parental strain. However, we observed a large proportion of eggs with undetectable or severely reduced *Wolbachia* titers laid by soma-infected mothers. In contrast, nearly all embryos laid by germline-infected mothers were robustly infected. FISH visualization of Wolbachia in ovaries revealed populations of uninfected ovarioles (the individual egg-producing structures) in soma-infected mothers, while all germline-infected and parental ovarioles were infected.

Conclusions

We have demonstrated that soma-to-germline Wolbachia spread is capable of facilitating vertical transmission in *Drosophila*; however, this route of transmission is insufficient to establish fully penetrant infection in all offspring. Consequently, uptake of *Wolbachia* into embryonic germ cells appears to be essential for robust vertical transmission. Further, we propose an underappreciated form of cell-to-cell spread – from the soma to the germline – may be important for facilitating vertical transmission by ensuring ovariole colonization.



P-13 Temporal dynamics of Anaplasma marginale infection in calves at the wildlife-livestock interface in the Mnisi communal area, Mpumalanga, South Africa

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Background
Bovine anaplasmosis, caused by *Anaplasma marginale*, is one of the most important tick-borne diseases of cattle in South Africa. Data collected in the study area of the Mnisi Community in the Mpumalanga Province indicate the presence of *A. marginale*, with occasional bovine anaplasmosis cases reported at villages close to the wildlifelivestock interface. The infection dynamics, and *A. marginale* strain diversity, during a 12-month period were examined in ten calves in a peri-urban area and a wildlife-livestock interface within the Mnisi community.

Method
Blood samples collected monthly from five calves each in each area were screened using an *A. marginale/A. centrale* duplex real-time PCR, *A. marginale* strain diversity was determined by *msp1*α genotype analysis and 16S rRNA microbiome analysis was conducted on blood samples taken at the last time point.

Results
The real-time PCR assay confirmed the presence of *A. marginale* in all five calves in the peri-urban area from the first month, but in only two calves at the wildlife-livestock interface and only after six months; this correlates with the occasional bovine anaplasmosis cases observed at the wildlife-livestock interface. Microbiome analysis detected *A. marginale* 16S rRNA sequences in the same calves, but nine of the ten calves also contained other *Anaplasma* sequences. *Msp1*α genotype analysis revealed 42 *A. marginale* genotypes in calves in the peri-urban area and 10 genotypes from the wildlife-livestock interface.

Conclusions
Methods of cattle management, acaricide treatment and cattle density could explain differences in exposure to *A. marginale* in the two areas. Our results revealed that most calves were superinfected by distinct *A. marginale* strains within the 12-month study period, indicating continuous challenge with multiple strains that should lead to robust immunity in the calves and endemic stability in the area. Cattle might benefit from cross-protection afforded by infection with other *Anaplasma* species.

P-14 Phenotypic Characterization of Coxiella burnetii isolates from different host species using tissue cell culture infection models

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Background

Coxiella (*C.*) *burnetii*, the etiological agent of the worldwide distributed zoonotic disease Q fever, causes different forms of disease in humans (acute, chronic) and ruminants (asymptomatic, fertility disorders). Genomic groups or genotypes are correlated with disease outcome and/or host. Besides, host factors have been proposed to influence disease manifestation and outcome.

Method

The aim of the project is to show isolate-specific differences in cell culture models resembling entry side (monocyte-derived macrophages THP-1) and replication niches (trophoblasts F3, udder cells PS). *C. burnetii* Nine Mile phase I and six field isolates of different host origin (human, sheep, goat, cattle) and disease outcome characterized phenotypically by replication and invasion rate as well as targeted gene expression.

Results

THP- 1 cells were permissive for Nine Mile I and isolates from sheep and goat with replications rates around 2 after 7 days but with invasion rates < 1% after 4h for the field isolates. Replication in F3 was higher for goat isolates compared to the other isolates. Invasion rates were low with < 3% for all isolates except for the cattle isolate with > 15%. PS were permissive for all isolates with replication rates of approximately 2 after 7 days except for the milk isolate. Invasion rates of approximately 5% were detected after 4h for most isolates with a maximum of > 10% after 4h. There was no correlation between replication and invasion.

Conclusions

Based on replication and invasion assays, we can detect phenotypic differences of the here tested isolates. Targeted gene expression analyses are ongoing. Isolates, which show the most significant differences, will be selected for proteome analysis. This might support the identification of potential factors and adaption mechanisms that could be relevant for the isolate-specific virulence.

P-15* **Dynamics of the immune responses to scrub typhus in non-human primate (Rhesus macaque) model and in human study**

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Background
Scrub typhus is a mite-borne rickettsiosis, which is treatable but potentially severe if untreated, or diagnosis is delayed/missed. In endemic areas, diverse strains of *Orientia tsutsugamushi*, the causative agent of scrub typhus, has been observed. However, how strain-specific immune responses and previous exposure contribute to the course of infection of remains unclear

Method
In this study, we investigated the immune responses in longitudinal samples in pediatric scrub typhus patients and Non-Human Primate model (Karp and Gilliam strain). The comparative analysis of the immune responses (cellular-, humoral- and cytokine-mediated immune responses) along with the clinical presentations were evaluated

Results
The comparative analysis demonstrated that the NHP model reassemble the clinical presentations in human scrub typhus. Clinical signs and immune responses with strain-specific characteristics were noticed in both Human and NHP studies. The most prevalent strain in human scrub typhus was UT76 (40%) followed by Ikeda (20%) Kato (6%) and TA716 (6%) strain. Kinetics of the specific humoral-, cellular- and cytokine-mediated immune responses have revealed similar pattern of the responses in the UT76-infected human and Karp-infected macaques while the other strains induced differences in the dynamic of the immune response pattern over a period of the observation.

Conclusions
The antigenic heterogeneity of *O. tsutsugamushi* has been a serious obstacle for developing effective diagnosis and a universal scrub typhus vaccine. Results in this study suggest a strain specific immune response presentation. More in-depth analysis could be enable to identify a critical Immunological markers correlate with the protective mechanism against Scrub typhus. Exploration of the conservative immunological markers between the NHP model and humans will be used to predict desired benefit for vaccine safety and immunogenicity study. Moreover, this is beneficial to find protection/appropriate clinical marker or endpoint in animal challenge studies and extrapolate to humans.

*Student paper

P-16 Rickettsia sibirica mongolitimonae. CRETAV experience

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Background

Rickettsia sibirica mongolitimonae infection was first described in Spain in 2008. Cases have been mainly published in the Mediterranean area. Clinical characteristics include fever, eschar, and sometimes, rash and lymphangitis. Due to this sign, it was called Lymphangitis-Associated-Rickettsiosis (LAR). Clinical spectrum has broadened, involving it as cause of encephalitis, myopericarditis, retinal vasculitis and septic shock. In Europe, *Hyalomma* and *Rhipicephalus pusillus* could be the vectors. Objective: Epidemiological/clinical/microbiological description of*R. sibirica mongolitimonae*-infected patients from San Pedro University Hospital (La Rioja) and other Centres in Spain, whose samples were processed at Center of Rickettsiosis and Arthropod-Borne Diseases (CRETAV).

Method

Review of *ompA/ompB/gltA* PCR amplicons of *R. sibirica mongolitimonae* at CRETAV in EDTAblood/biopsies/swabs.

Results

Twenty-three patients (16 men/7 women) from La Rioja (6)/Aragón (7)/Andalucía (4)/Comunidad Valenciana (3) and Madrid (3) were described (2007-2022). *R. sibirica mongolitimonae* was amplified in 18 swabs/4 biopsies/2 eschar punctures/2 EDTA-blood samples. Median age: 65 years (range:5-82). All patients were attended between March-September (7 cases in June). Only 3 (15%) remembered a tick-bite. All patients had fever. Twenty of them (87%) developed unique (15) or multiple (5) eschar. Tick-bite was located on: lower limbs (7)/gluteus (3)/forearm (3)/head (3)/groin (1)/hip (2) and scrotum (1). Lymphangitis was detected in 9 (40%), and maculopapular rash, in 10 patients. A patient with fever, a necrotic gluteus eschar and enlarged inguinal lymph nodes developed myopericarditis. Another patient with fever and rash had a septic-shock. All received doxycycline and recovered.

Conclusions

1. *Rickettsia sibirica mongolitimonae* causes a broad spectrum of clinical manifestations that should be considered in tick-exposed patients and/or in those presenting fever and eschar in Spain.
2. Eschar, with/without rash, with/without lymphangitis, is the main sign that should guide diagnosis.
3. LAR acronym only refers to 40% infected patients.
4. Eschar swabs are the best sample (non-invasive and highly effective) for the molecular diagnosis.

P-17* **Chlamydiosis: a neglected zoonotic disease in Costa Rica**

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Background

The great diversity of wildlife of Costa Rica allows diverse transmission cycles of zoonotic chlamydial species. The present work aims to inform about the detection and characterization of chlamydial species in mammals and birds, as well as to document zoonotic cases in our country.

Method

Individual or pooled tracheal swabs, cloacal swabs, feces or lung tissues from asymptomatic or clinical diseased animals were analyzed using molecular techniques (qPCR and conventional PCR, amplifying *ompA* gene, 23S/16S rDNA genes, and sequencing). Positive samples underwent also isolation in cell culture.

Results

Chlamydia psittaci genotype A was found infecting different wild birds living in captivity (symptomatic green macaws [*Ara ambiguus*], one symptomatic scarlet macaw [*Ara macao*], one symptomatic green heron [*Butorides virescens*]), pet animals in homes (one symptomatic cockatiel [*Nymphicus hollandicus*], one asymptomatic canary, and a symptomatic cat), as well as commercial and backyard chickens, meanwhile*, Chlamydia psittaci* genotype B was found in a feral pigeon (*Columba livia*) and a wild pigeon (*Columba flavirostris*) found dead in public parks. For the first time in Central America, *Chlamydia gallinacea* was determined in asymptomatic backyard chicken; *Chlamydia pneumoniae* in asymptomatic snakes (*Boa constrictor*) in a herpetological exhibition center, and *Chlamydia suis* in pigs with conjunctivitis in a farm, representing a risk for the people who live and handle with them. Finally, the presence of a not yet well characterized *Chlamydia* species (it may be a new species) was detected in an African hedgehog (*Atelerix albiventris*) employed as a pet. Isolation in cell culture was possible in four cases. Finally, three cases of possible psittacosis were documented with clinical manifestations. In one, *C. psittaci* genotype A was detected.

Conclusions

The results confirm the need to implement control and prevention measures to avoid transmission between animals and towards people. *Chlamydia* spp. diagnosis should be implemented in health centers in Costa Rica.

*Student paper

P-18 **Molecular evidence of Chlamydia abortus in an oral swab during a Covid-19 screening in a refugee camp setting**

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Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of the coronavirus disease 2019 (COVID-19), causing a high rate of morbidity and elevate mortality all over the world. During an outbreak of COVID-19, occurred in a refugee camp sited in Southern Italy, we tested a total of 48 oral swabs for the detection of SARS-CoV-2. DNA was extracted from the same swabs for the research of other two zoonotic agents such as *Coxiella burnetii* and *Chlamydia* spp. that may cause disease with the same unspecific symptoms of SARS-CoV-2.

Method

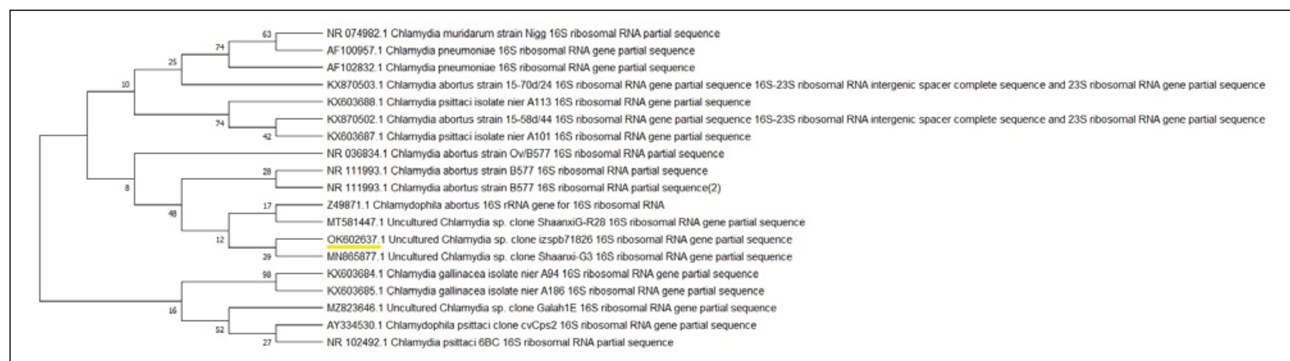
The presence/absence of DNA of *C. burnetii* and *Chlamydia* spp., was verified using two qPCR assays targeting *IS1111* and *16S* gene respectively. Amplicons of the periplasmic oligopeptide-binding protein gene (*OppA*) and of the 16S ribosomal RNA gene (*16S*) of *Chlamydia* spp. has been also amplified using a conventional PCR, sequenced by Sanger method and subsequently, a phylogenetic tree has been constructed using the MEGA6 software.

Results

Among the 48 analyzed DNAs, one sample was found to be positive for *Chlamydia* spp., while *C. burnetii* was not detected in any of the tested samples. The nucleotide sequence showed respectively 99.79% and 99.75% of homology with *OppA* and *16S* genes of *Ch. abortus*. Phylogenetic analysis confirmed that our sequence is grouped within a representative cluster constituted by *Ch. abortus.*

Conclusions

Chlamydiae comprise a group of zoonotic obligate intracellular organisms able of causing severe forms of disease in animals and humans. Among these, *Ch. abortus* is considered cause of abortion and fetal loss in livestock, but is also able to cause pneumonia in humans. Usually, human infections are the result of the exposure to infected domestic mammals. Our result suggests to verify the presence of these zoonotic agents, especially in small ruminants' farmers or in people who are in strict contact with these animals.



P-19 The occurrence of chlamydiae in German holdings of South American camelids

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Background
South American camelids (SAC) are kept in Europe in increasing numbers for wool-production, leisure activities, therapy, landscape conservation or breeding. Although they often live in close contact to humans and livestock, little is known about zoonotic and epizootic agents that could affect their health or reservoir status. Since chlamydiae are important endemic pathogens residing in the reproductive or gastrointestinal systems of the closely related ruminants, they were targeted in a systematic investigation of llamas and alpacas in ten representative holdings crossover Germany.

Methods
Faecal and blood samples were obtained from 295 healthy animals (195 alpacas and 100 llamas) during a project supported by the Federal Ministry of agriculture. Faecal samples were screened for Chlamydiaceae by a stepwise real time PCR approach, and circulation of Chlamydia-specific antibodies was investigated by means of two different ELISAs.

Results
Shedding of chlamydiae was detected in eight of ten SAC holdings and in 26.8% of animals (n=79, 95% CI 22.0-32.2%). The positivity rate was similar in llamas (28.0%) and alpacas (26.2%). Typing with species-specific PCR identified *Chlamydia (C.) suis* as the predominant species with 57.0% of *Chlamydiaceae*-positive samples, followed by *C. pecorum* with 17.7%. 26.6% of samples could not be typed, probably due to low DNA-content. Two isolates were recovered from a flock with high chlamydial loads (Cq values 22) and further characterized by sequencing of a 1900 bp fragment of the 23S-rRNA gene. Phylogenetic analysis revealed that the isolates clustered in the *C. trachomatis/C. suis* clade, but apart from porcine *C. suis* isolates. *Chlamydiaceae*-specific antibodies were detected in the eight PCR-positive holdings.

Conclusions
Our findings suggest that South American camelids are carriers of chlamydiae and that a C. suis-like agent predominates in faecal shedding. Its pathogenic and zoonotic impact remains to be elucidated.

P-20* Prevalence of new and established avian chlamydial species in humans and their psittacine pet birds in Belgium

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Background

C. psittaci is a recognized zoonotic respiratory pathogen of pet birds. *C. avium* and *C. gallinacea* are recently discovered *Chlamydia* species with an unknown zoonotic potential and unknown prevalence rate in psittacine birds. *C. abortus* has occasionally been detected in pet birds and just as *C. psittaci*, this species can be transmitted to humans. Whether these new species pose a threat to psittacine birds and their owners, is unclear. Therefore, the prevalence and zoonotic potential of the four species was examined in the Belgian psittacine pet bird population.

Method

Samples were collected by volunteering pet bird owners across Belgium. Each volunteer received a package by regular mail containing a questionnaire and the necessary material to collect 4 avian fecal swabs and 2 human pharyngeal swabs. Subsequently, all samples were examined with a genus-specific and four species-specific PCR assays in combination with culture.

Results

This study confirmed that *Chlamydiaceae* are still very prevalent in Belgian psittacine birds, with a prevalence of 39.3% (33/84). The predominant part of the infections could be attributed to *C. psittaci* (26.2%, 22/84) and most strains were genotyped as the virulent genotype A. This species was also found in 18.2% (4/22) of the bird owners, indicating the transmission from birds to the owners. In birds, *C. avium* showed a prevalence of 13.1% (11/84), but transmission to the owners was not detected. *C. gallinacea* and *C. abortus* were not detected in this study.

Conclusions

This study demonstrates that *C. psittaci* genotype A is still actively circulating in pet birds and is regularly transmitted to humans. Moreover, the emergence of *C. avium* is illustrated whereas in earlier studies, the agent has only sporadically been detected in psittacine birds.

*Student paper

P-21* **Coxiella burnetii as an expression vector for SARS-CoV-2 spike protein: a tool for the study of host-pathogen interactions**

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Background

Coxiella burnetii is a Gram-negative obligate intracellular pathogen that causes Q fever, a highly variable human disease that typically manifests as an acute flu-like illness. While *C. burnetii* whole cell vaccination (WCV) induces potent protection against Q fever, there is limited data indicating that *C. burnetii* vaccination may elicit non-specific immune protection against distinct pathogens.

Method

Prior to studying this *in vivo*, we aim to expand the repertoire of experimental tools to study *C. burnetii*-host immune interactions, knowledge of which is currently lacking. Specifically, the Nine Mile II (NMII) strain of *C. burnetii* was genetically engineered to constitutively express the S1 subunit of the SARS-CoV-2 spike protein on outer membrane protein, CBU0307, with the aim of using it as a control for studies of host-pathogen immune interactions. The S1 subunit, alone, is sufficient to elicit SARS-CoV-2 immunogenicity as it contains the receptor binding (RBD) used by the virus to enter human cells via interaction with ACE2 receptors.

Results

Expression of the S1 subunit on CBU0307 was driven by p1169, a constitutively active *C. burnetii* promotor and directed to the outer membrane protein using the CBU0307 signal sequence. *C. burnetii* S1 expression was confirmed first via immunoblot of both whole cell lysates and outer membrane fractions with SARS-CoV-2 spike and RBD polyclonal antibodies. Further confirmation of expression was observed via immuno-electron microscopy.

Conclusions

While immunogenicity is yet to be confirmed, these data mark the first report of non-native protein expression on *C. burnetii*, to our knowledge. This tool will not only aid in our future investigation of the potential nonspecific immune effects of *C. burnetii* vaccination, but it will also allow us to evaluate the feasibility of *C. burnetii* as a vaccine vector.

*Student paper

P-22 Post-mortem detection of Coxiella burnetii in eight ewes from a flock recently confronted with Q fever clustered human cases sampled 89 to 229 days post-lambing

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Background

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*. Ruminant species provide a major primary reservoir of exposure for humans. Bacterial shedding occurs in vaginal fluids and feces of infected sheep, secondarily contaminating bedding areas and other indoor and outdoor environments . Shedding is known to be massive by clinically affected herds. However, knowledge on tissue tropism in individual ewes remains scarce.

Method

Focusing on a flock of 500 sheep recently confronted with Q fever abortion and an alert of clustered human cases, we investigated the tissue dissemination of *C. burnetii* in eight non-aborting ewes out of the 58 ewes planned to be culled for zootechnical reasons. The time-frame between lambing and necropsy ranged from 89 to 229 days. A serological test was performed and diverse tissues, swabs and body fluids were collected for examination by histo-pathology and screening by qPCR for the presence of *C. burnetii* DNA based on a qPCR assay targeting the IS1111 insertion sequence.

Results

Histological investigations did not reveal any microscopic lesion relevant to diagnostic purposes. Low levels of *C. burnetii* DNA were detected in one to six tissue samples depending on the considered ewe. The most frequently positive samples were nasal and vaginal swabs; the tissues found positive were the cardiac valves, mammary lymph node, uterus, ovary, bladder, Peyer's patch and bone marrow; DNA was also detected in a milk sample and a serum sample.

Conclusions

This study reveals that *C. burnetii* DNA may still be detected in diverse tissues and body fluids of individual ewes a long time after lambing. These results in sheep are consistent with previous data from experimental infection in goats. Studies should be undertaken on more individuals to assess the distributions according to their clinical and excretory histories.

P-23 Evaluation of diagnostic accuracy of VirClia Lotus for the detection of Coxiella burnetii infections

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Background

Our laboratory of serology recently introduced the VirClia Lotus instrument for the screening of *Coxiella burnetii* infection. The suspicion of false negative results possibly caused by a prozone effect lead to a deep investigation of the automaton performance characteristics.

Method

A collection of 193 serum samples collected at Lausanne University Hospital (Chuv) were screened for the detection of *C. burnetii* phase I and phase II antibodies with the VirClia Lotus instrument. All sera were tested in native and after a 1/5 dilution. Sera showing at least one positive result were tittered with the reference method, indirect immunofluorescence assays (IFA).

Results

Results showed a sensitivity rate of 97% and a false negative rate of 1.05% when using natives sera for the detection of IgM phase II and 1/5 diluted sera for the detection of IgG phase II. Importantly, 11 sera exhibited a prozone effect – negative results for native serum and positive result after 1/5 dilution- on IgG phase II antibodies at the screening. VirClia Lotus is a performant screening system for *C. burnetii* infection.

Conclusions

Our analyses suggests that to avoid a prozone effect (representing 5.7% of our collection), sera should be diluted at 1/5 prior IgG phase II investigation. Additionally, this study pointed out an important alarm ("z-max in sample tube") of the automaton that might cause false negative results.

P-24 Coxiella and Rickettsia spp. in ticks collected from Otolemur crassicaudatus in South Africa

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Background

Nonhuman primates are responsible for zoonosis, some of them pandemic. This study aimed to investigate microorganisms of ticks collected from thick-tailed bushbabies (*Otolemur crassicaudatus*) in South Africa.

Method

During 2014-2019, bushbabies were inspected for tick collection. Most immature specimens were pooled according to the species (classified by morphological taxonomy) and stage. Nucleic acid extracts of individual specimens and pools were used as templates for PCR screening of *Coxiella*, *Rickettsia*, Anaplasmataceae, *Borrelia*, *Spiroplasma* and *Babesia/Theileria* spp. Genetic characterization of tick species was also performed from individual ticks and pools using PCR assays targeting the 16S-RNA gene.

Results

Ticks belonging to *Haemaphysalis* and *Rhipicephalus* genera (n=135) from 44 bushbabies were studied. The classification at the species level was not achieved for all the specimens despite the morphologic and genetic analysis (table). Nucleotide sequences analysis from ticks suggests that sibling *Haemaphysalis* species and mixture of species in larvae pools might have occurred. *Coxiella* endosymbionts were detected in all but five samples. The analysis of the *rpoB* sequences showed five different *Coxiella* genotypes. All but those amplified from *R. simus* and *R. appendiculatus* showed <90% identity with previously known isolates (table). One pool showed infection with *Rickettsia* sp. The obtained *ompA* and *ompB* fragment genes were homologous to those from a *Rickettsia* found in rodents in South Africa. The *sca4* fragment gene showed the highest identity with *R. heilongjiangensis* (99.4%). Anaplasmataceae, *Borrelia*, *Spiroplasma* and *Babesia/Theileria* spp. were not detected.

Conclusions

These data demonstrates the bushbaby infestation with *Haemaphysalis* and *Rhipicephalus*, tick species harboring endosymbionts such as *Coxiella* sp. New *Coxiella* isolates have been detected in *Haemaphysalis* spp. The role of these tick species as vectors of *Rickettsia* spp. should be better investigated. This study shows the need of a thorough taxonomical study of bushbabies ticks, including molecular characterization, as morphological identification of fed larvae is tricky.

Table: Studied ticks and the associated microorganisms

Sample	No. of ticks	Stage	Tick identification		Microorganisms [Identities; %- GenBank Accession no.]	
			Morphologic	Genetic [Identities; %- GenBank Accession no.]	*Coxiella*	*Rickettsia*
1	1	L	NI	*R. simus* [100-KJ613641]	NA	NA
2	3	L	*Haemaphysalis* spp.	*Haemaphysalis* sp. (genotype 1a) [92.2-NC_058312]	NA	NA
3	1	L	*Haemaphysalis* sp.	*Haemaphysalis* sp. (genotype 1b) [91.9-NC_058312]	US	NA
4	2	L	*H. leachi*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][1]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
5	1	N	*H. leachi*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][1]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
6	3	L	*R. near warburtoni*	*Haemaphysalis* sp. (genotype 1a) [92.2-NC_058312]	NA	NA
7	12	L	*R. warburtoni*	*Haemaphysalis* sp. (genotype 1c)[2] [90.8-NC_058312]	*Coxiella* sp. (genotype 2) [89.8-KY677983]	NA
8	13	L	*R. warburtoni*	US; *R. simus* [100-KJ6136413][3]	US	*Rickettsia* sp. [100-KT835150,KT835128; 99.4- AP019863]
9	1	F	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
10	1	F	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1b) [88.4- KP985315]	NA
11	1	M	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
12	1	M	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1b) [88.4- KP985315]	NA
13	1	M	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
14	1	M	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
15	1	M	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
16	1	M	*H. zumpti*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
17	2	N	*H. zumpti*	*Haemaphysalis* sp. (genotype 2b) [96.5-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
18	1	F	*H. elliptica*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
19	1	F	*H. elliptica*	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
20	1	F	*H. spinulosa* like	*Haemaphysalis* sp. (genotype 2b) [96.5-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
21	1	M	*H. spinulosa* like	*Haemaphysalis* sp. (genotype 2a) [96.3-KJ613637][4]	*Coxiella* sp. (genotype 1a) [88.8- KP985315]	NA
22	6	L	*R. sanguineus* s.l.	*R. simus* [100-KJ613641]	*Coxiella* sp. (genotype 3) [99.8-LC634960][6]	NA
23	6	L	*Haemaphysalis* spp.	*Haemaphysalis* sp. (genotype 1d) [92.4-NC_058312]	*Coxiella* sp. (genotype 4) [89.8-KY677983]	NA
24	21	L	*H. aciculifer*	*Haemaphysalis* sp. (genotype 1d)[5] [92.4-NC_058312]	US	NA
25	23	L	*H. aciculifer*	*Haemaphysalis* sp. (genotype 1d)[5] [92.4-NC_058312]	US	NA
26	23	L	*H. aciculifer*	*Haemaphysalis* sp. (genotype 1d)[5] [92.4-NC_058312]	*Coxiella* sp. (genotype 4) [89.8-KY677983]	NA
27	1	N	*R. appendiculatus*	*R. appendiculatus* [100-KC503257]	*Coxiella* sp. (genotype 5) [99.8-LC634931][7]	NA
28	3	L	*R. simus*	*R. simus* [100-KJ613641]	NA	NA
29	1	L	*Rhipicephalus* sp.	*R. simus* [100-KJ613641]	*Coxiella* sp. (genotype 3) [99.8-LC634960][6]	NA
30	1	L	*Rhipicephalus* sp.	*R. simus* [100-KJ613641]	*Coxiella* sp. (genotype 3) [99.8-LC634960][6]	NA

L: Larvae; N; Nymph; F: Female; M: Male; NI: Not identified; NA; Not amplified; US: Unclear sequences; *H.*: *Haemaphysalis*; *R.*: *Rhipicephalus*

[1]: The highest identity reached with available sequence in GenBank of *H. leachi* (MN661151) was 89.36%

[2]: The sequence is not perfect and more than one tick species could be amplified

[3]: Inconclusive sequences, more than one species could be amplified. Two individuals were removed from the pool, washed and a PCR assay showed 100% identity with *R. simus*

[4]: There are not available sequences of *H. zumpti* in GenBank

[5]: The highest identity reached with available sequence in GenBank of *H. aciculifer* (LC634549) was 90.32%

[6]: Sequence obtained from *R. simus* specimens

[7]: Sequence obtained from *R. appendiculatus* specimens

P-25 Detection of intracellular bacteria and Hepatozoon martis in ticks collected from minks in Spain

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Background

European mink (*Mustela lutreola*; EM) is critically endangered small mustelid, threatened mainly because of invasive American mink (*Neovison vison*; AM). Wildlife is an important source of emerging zoonotic agents, including vector-borne ones. Aim: To investigate ticks from mink (EM/AM) and their associated microorganisms.

Method

From 2012 to 2021, a total of 591 ticks were collected from minks in northern Spain (figure). Ticks were classified and pooled according to the species, stage, host and preservation method (frozen/ethanol). Samples were PCR-screened for *Anaplasma/Ehrlichia/Neoehrlichia*, *Borrelia*, *Rickettsia*, *Coxiella/Rickettsiella/Francisella*, *Spiroplasma*, *Trypanosoma* and *Babesia/Theileria/Hepatozoon*. Pools from frozen ticks were also analyzed for Flaviviridae, Phenuiviridae and Orthonairoviridae viruses.

Results

All ticks but one *Rhipicephalus sanguineus* s.l. belonged to the *Ixodes* genus; *Ixodes hexagonus* (n=466;61 pools) and *Ixodes acuminatus* (n=124;16 pools) (table). *Ehrlichia* sp. (related to *Ehrlichia* sp. HF) and '*Candidatus* Neoehrlichia mikurensis' (related to Asian strains) were amplified from 8/16 and 4/16 *I. acuminatus* pools, respectively. *Rickettsiella* sp. (previously found in *I. trianguliceps* and *I. ventalloi*) was detected in *I. acuminatus* (n=6) and *I. hexagonus* (n=3) pools. *Coxiella* sp. was confirmed in all but seven *Ixodes* pools showing similar sequences (related to those from *Ixodes* spp.) in *I. hexagonus* and *I. acuminatus*, and a different genotype in *R. sanguineus* s.l. (related to those from this tick species). *Hepatozoon martis* was found in four *I. hexagonus* pools. Remaining studied bacteria, protozoan and viruses were not detected.

Conclusions

This is the largest study of ticks from minks and their associated microorganisms. *Ehrlichia*, '*Ca.* N. mikurensis', *Rickettsiella* and *Coxiella* spp. are reported for the first time in *I. acuminatus*. The role of this species as vector of the human pathogens '*Ca.* N. mikurensis' and *Ehrlichia* sp. should be investigated. *Coxiella* sp. is a common endosymbiont of the studied ticks. Our results suggest that *I. hexagonus* could be a potential vector of *H. martis*.

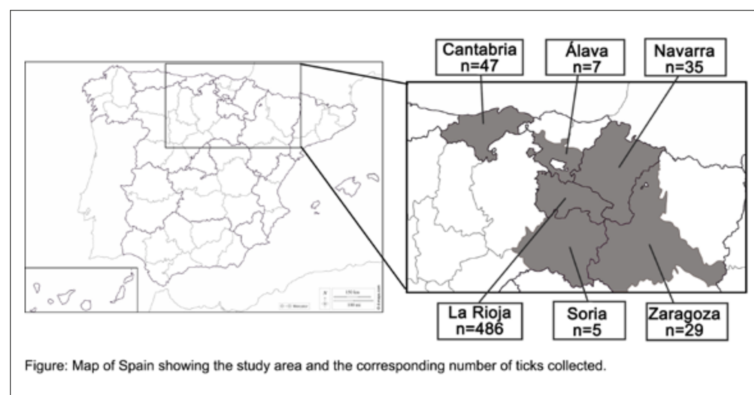

Figure: Map of Spain showing the study area and the corresponding number of ticks collected.

Table: Ticks analyzed in this study.

Tick species	Preservation	Stage	Host	No. of ticks	No. of pools
I. hexagonus	Frozen	Larva	EM	24	3
		Nymph		119	8
		Female		65	14
		Male		1	1
		Nymph	AM	36	3
		Male		1	1
		Female		44	8
	Ethanol	Nymph	EM	101	8
		Female		45	11
		Nymph	AM	19	2
		Female		11	2
Total *I. hexagonus*				**466**	**61**
I. acuminatus	Frozen	Female	EM	60	7
		Female	AM	4	2
	Ethanol	Female	EM	36	4
		Female	AM	24	3
Total *I. acuminatus*				**124**	**16**
***R. sanguineus* s.l.**	Ethanol	Male	EM	**1**	**1**
Total				**591**	**78**

I.: *Ixodes*; *R.*: *Rhipicephalus*; EM: European mink (*Mustela lutreola*); AM: American mink (*Neovison vison*)

P-26* Simple clinical and laboratory predictors to improve empirical treatment strategies in areas of high scrub typhus and dengue endemicity, central Vietnam

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Background

Dengue fever (DF) is highly endemic in Vietnam, but scrub typhus (ST)-although recognized as an endemic disease-remains underappreciated. These diseases together are likely to account for more than half of the acute undifferentiated fever burden in Vietnam. ST is a bacterial disease requiring antimicrobial treatment, while DF is of viral etiology and does not. Diagnostic access and current understanding of empirical treatment strategies for both illnesses remain limited. In this study, we aimed to contribute to the clinical decision process in the management of these two important etiologies of febrile illness in Vietnam.

Method

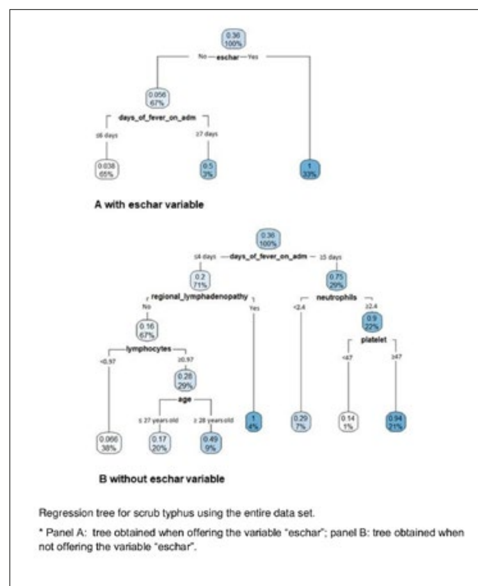
Using retrospective simple clinical and laboratory data from 221 PCR-confirmed ST cases and 387 NS1 protein positive DF patients admitted to five hospitals in Khanh Hoa province, we developed models to discriminate ST from DF using multivariable logistic regression (MLR) and classification and regression trees (CART).

Results

Using M-LR, following seven predictors were identified, that reliably differentiate ST from DF; eschar, regional lymphadenopathy, an occupation in nature, increased days of fever on admission, increased neutrophil count, decreased ratio of neutrophils/ lymphocytes, and age≥40. Sensitivity and specificity of predictions based on these seven factors reached 93.7% and 99.5%, respectively. When excluding the “eschar” variable, the values dropped to 76.3% and 92.3%, respectively. The CART model generated one further variable; increased days of fever on admission, when eschar was included, the sensitivity and specificity was 95.0% and 96.9%, respectively. The model without eschar involved the following six variables; regional lymphadenopathy, increased days of fever on admission, increased neutrophil count, increased lymphocyte count, platelet count ≥47G/L and age≥28 as predictors of ST and provided a sensitivity of 77.4% and a specificity of 90.7%.

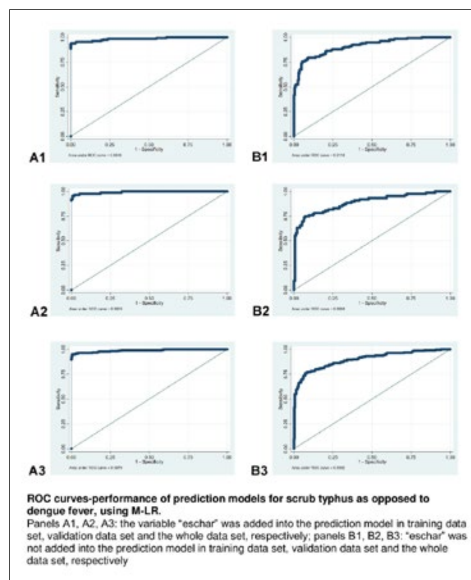
Conclusions

The generated algorithms contribute to differentiating ST from DF using basic clinical and laboratory parameters, supporting clinical decision making in areas where ST and DF are co-endemic in Vietnam.



Regression tree for scrub typhus using the entire data set.

* Panel A: tree obtained when offering the variable “eschar”; panel B: tree obtained when not offering the variable “eschar”.



ROC curves-performance of prediction models for scrub typhus as opposed to dengue fever, using M-LR.
Panels A1, A2, A3: the variable “eschar” was added into the prediction model in training data set, validation data set and the whole data set, respectively; panels B1, B2, B3: “eschar” was not added into the prediction model in training data set, validation data set and the whole data set, respectively

	Clinical manifestations			Routine hematological blood laboratory			Clinical manifestations & Routine hematological blood laboratory		
	aOR	95%CI OR	P-value	aOR	95%CI OR	P-value	aOR	95%CI OR	P-value
Eschar (no using)									
Regional lymphadenopathy	96.3	12.2-759	**<0.001**				78.2	9.20-665	**<0.001**
An occupation in nature	3.75	2.02-6.96	**<0.001**				3.87	1.89-7.91	**<0.001**
Age over 40	3.39	1.78-6.46	**<0.001**				3.94	1.94-8.01	**<0.001**
Days of fever on admission (Nr)	1.49	1.30-1.71	**<0.001**				1.42	1.22-1.66	**<0.001**
Neutrophil count				2.09	1.75-2.50	**<0.001**	1.89	1.54-2.32	**<0.001**
Ratio of N/L (neutro/lymph)				0.61	0.53-0.71	**<0.001**	0.68	0.57-0.81	**<0.001**
	AUC	**95% CI**		**AUC**	**95% CI**		**AUC**	**95%CI**	
ROC –analysis (n=364)	0.862	0.823-0.896		0.831	0.790-0.869		0.912	0.878-0.939	

*Student paper

P-27 Search for Parasitation of Trichoptera, Sericostoma spp., by Rickettsia and alike organisms

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Background

Insects are likely to play an important role in the epidemiology of various diseases. The potential role of aquatic insects in a zoonotic cycle of Rickettsiae has not been previously investigated. Because aquatic insects act as intermediate hosts, they also may act as vectors of Rickettsia like organisms. The caddisflies, or order Trichoptera, are a group of insects with aquatic larvae and terrestrial adults.

Method

Aiming to make a pre-screening of, we have collected 4 samples of larvae of aquatic insect, caddisfly Sericostoma *spp*, order Trichoptera, from locality of "Low Tatras". Following the isolation of DNA, we have tested the positivity of these insects, by standard PCR using universal and species specific genes for: *Anaplasma spp* (*23S*), *Wolbachia spp* (*16S*), *Rickettsia spp* (*Ompa*, *RKND03*), *Bartonella spp* (*ITS2*), and *Borrelia spp* (*16S/ITS4*).

Results

A molecular biological approach referred to above, has revealed one Anaplasma *spp*. positive case, and two has appeared to be infected with Rickettsia like organisms. A verification of the results was completed by sequencing analysis. Surprisingly the two Rickettsia like sequences has produced no alignments with any of the known Rickettsia *spp*. Conversely, a significant alignment (96% query cover) was perceived with a Gemmataceae bacterium PX52 (accession number CP042425.1). The family Gemmataceae accommodates aerobic, chemoorganotrophic planctomycetes, which inhabit various freshwater ecosystems, wetlands and soils.

Conclusions

This study demonstrates a broad intermediate host range for Trichoptera that may possibly act as vector for various bacteria. Our followers were too small in numbers to make a statistically significant conclusion. Bearing in mind the attractiveness of the bacterial underwater world in flows, additional studies are needed to negate or confirm the appearance of Rickettsia like or related organisms in aquatic domain. Funding: This study was supported by the IHU Méditerranée Infection, France; and by the Slovak agencies: SRDA-19- 0066, and the VEGA: 2/0010/19.

P-28 **Chemical comparison of lipopolysacharides of intracellular bacteria**

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Background

Lipopolysaccharide (LPS), also known as endotoxin, is an essential component of the outer membrane of almost all gram-negative bacteria. This polymer is composed of three major domains: the lipid moiety, termed lipid A, the core oligosaccharide, and the O-side-chain polysaccharide. Rickettsiae are strictly intracellular gramnegative bacteria that have the common feature of being spread by arthropod vectors; therefore they are exposed to hard environment conditions. It is known that rickettsial LPSs are capable to display noticeable antigenic activity and elicit inflammatory host response. Despite the wide knowledge about rickettsial LPS, the immunoreactive epitopes from this macromolecule are poorly characterized in some rickettsial pathogens. In this study, we examined the LPS from *Rickettsia akari* (Transitional group) and *Rickettsia prowazekii* (Typhus group), the etiologic agent of Rickettsialpox and Epidemic typhus, respectively.

Method

LPS was extracted by the hot phenol-water approach, samples were subjected to Gas Chromatography mass spectrometry (GC-MS). Lipid A was isolated using mild acid hydrolysis and analyzed by GC-MS to compare the fatty acid composition of both rickettsial species.

Results

Analysis of both rickettsial LPS split by total acid hydrolysis revealed significant divergence in the concentration of neutral sugars. Ribose and glucose were the most abundant monosaccharides in *R. akari* LPS, while the concentration of mannose was twofold higher in *R.prowazekii*. The neutral sugar fucose was detected in *R. akari* LPS only. Interestingly, no heptose and galactosamine were found, which are common sugar constituents of *Coxiella burnetii* LPS. We found palmitic and stearic acid as the main components of lipid A, moreover higher concentration of both fatty acids was observed in *R. prowazekii* lipid A.

Conclusions

In conclusion: similarity in composition of both rickettsial LPS was found. Differencies in sugars and fatty acid concentrations between both rickettsiae were observed. This study was supported by grant agencies: VEGA: 2/0057/19, APVV 19-0519 and APVV 19–0066.

P-29* Calcium signaling involvement in spotted fever group rickettsiae induced microvascular endothelial cell barrier permeability

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Background

Spotted fever group rickettsiae (SFGR) are obligate intracellular bacteria that can cause severe disease. These ATP parasites primarily infect microvascular endothelial cells (MEC) resulting in increased vascular permeability (VP), the major pathophysiological effect of SFG rickettsioses for which mechanisms are not fully elucidated. In physiologic and pathophysiologic conditions, MEC VP is linked to increased intracellular calcium concentrations [Ca2+]i, regulated by ATP-dependent calcium channels. Using *Rickettsia parkeri* (*RP*)-infected human brain MEC (HBMEC) *in vitro*, calcium chelators (DP-b99, DP-460) and the calcium channel blocker benidipine (but not nifedipine) abrogate *RP*-induced MEC barrier permeability (Figure 1). Benidipine blocks L-, N-, and T-type voltage-gated calcium channels (VGCC); however, how benidipine affects HBMECs is also unclear. Thus, we hypothesize that SFGR modulate specific MEC calcium transport systems leading to barrier dysfunction prior to direct rickettsial MEC damage.

Method

Using the Ca2+-sensitive dye Fluo-4-AM, we demonstrated increased [Ca2+]i in *RP*-infected HBMECs correlating with decreased HBMEC barrier permeability detected by electric cell-substrate impedance sensing. Additionally, through bioluminescence assays, we showed that *RP*-infection decreased HBMEC ATP levels kinetically associated with decreased HBMEC barrier permeability.

Results

Notably, benidipine abrogated *RP*-induced HBMEC [Ca2+]i increases and ATP decline corresponding to delayed *RP*-induced HBMEC barrier dysfunction. To address calcium channel involvement, RNA-seq demonstrated that HBMECs basally express transcripts encoding pore-forming alpha subunits for specific L-(Cav1.3) and T-(Cav3.1) type VGCCs, including CACNA1D and CACNA1G as well as those which encode auxiliary VGCC beta, alpha-delta, and gamma subunits: CACNB1, CACNB3, CACNB4, CACNA2D1, CACNA2D2, CACNG6, CACNG7, and CACNG8 (Figure 2). Notably, HBMECs 50h post *RP*-infection had significantly reduced CACNA1G (-1.674 log2) and CACNA2D2 (-1.159 log2) expression.

Conclusions

SFGR-induced MEC permeability involves reduced cellular ATP, consequently increased [Ca2+]i leading to increased HBMEC barrier dysfunction perhaps related to benidipine-sensitive T-type VGCCs that suggest rickettsia-specific channelopathy with VP pathogenesis.

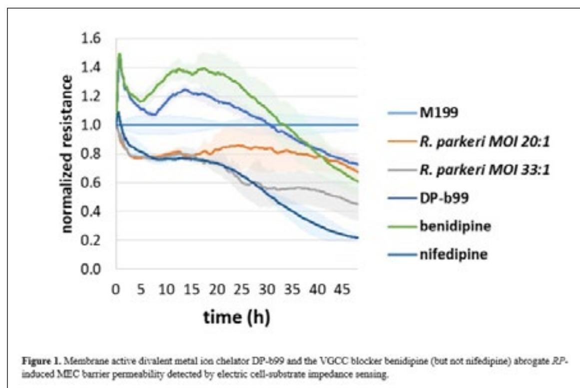

Figure 1. Membrane active divalent metal ion chelator DP-b99 and the VGCC blocker benidipine (but not nifedipine) abrogate *RP*-induced MEC barrier permeability detected by electric cell-substrate impedance sensing.

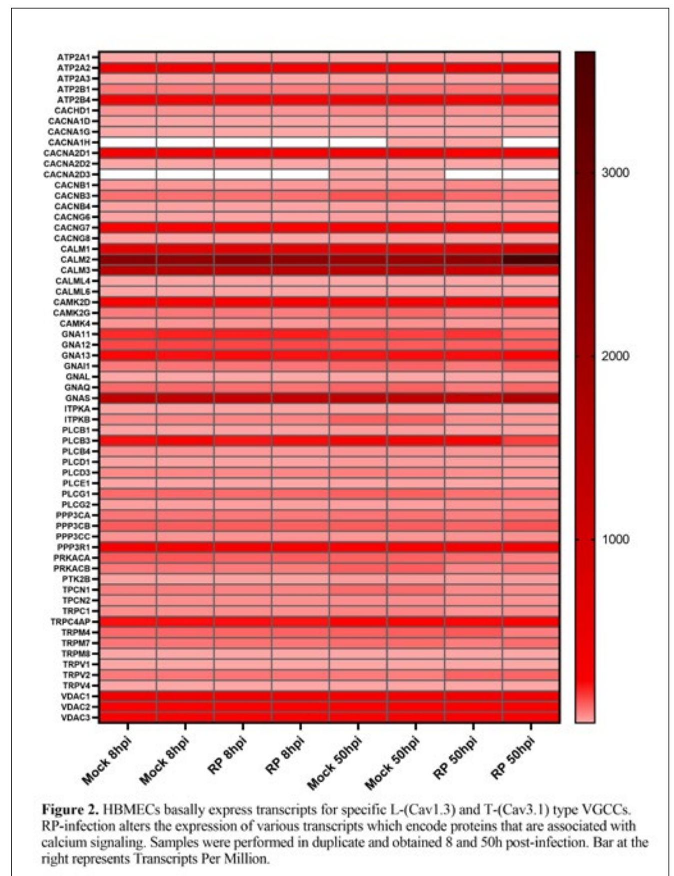

Figure 2. HBMECs basally express transcripts for specific L-(Cav1.3) and T-(Cav3.1) type VGCCs. RP-infection alters the expression of various transcripts which encode proteins that are associated with calcium signaling. Samples were performed in duplicate and obtained 8 and 50h post-infection. Bar at the right represents Transcripts Per Million.

*Student paper

P-30* Temporal Dynamics and Performance of PCR and Serological Diagnostic Tests for Scrub Typhus – in Khanh Hoa, Central Vietnam

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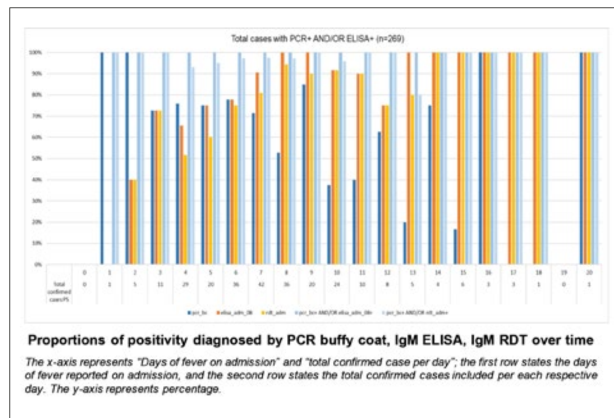
Background
Scrub typhus (ST) is an important treatable aetiology of acute undifferentiated fever in Vietnam. Lifethreatening complications are common among ST patients with diagnosis and treatment delays. Severe ST is reported with an estimated untreated mortality of 6-8%. In Vietnam, the proportion of complicated cases is approximately 15%. Timely diagnosis is crucial, but often complicated by a limited availability of tests and incomplete understanding of the bi-phasic disease dynamics. Here we present the temporal dynamics and performance for PCR-and serological-based assays in early diagnosis of ST in Vietnam

Method
Active hospital surveillance was established for ST in Khanh Hoa, Vietnam from January, 2013 to December, 2014 and from August, 2018 to March, 2020. For a total of 437 suspected ST patients, whole-blood and eschar swab specimens were collected, and diagnosed using PCR eschar, PCR buffy coat, ELISA IgM (cutoff ≥ 0.8), and RDTs.

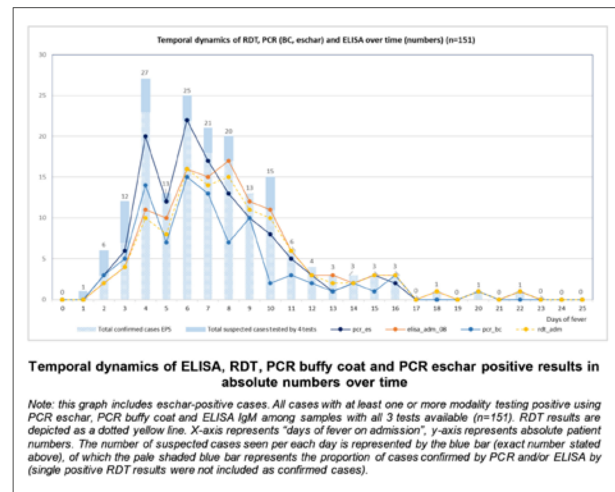
Results
A total of 1,038 samples from 437 suspected patients were collected. Of these, 421/437 (96.3%) cases were confirmed by PCR buffy coat, ELISA IgM, and 178/437 (40.7%) had PCR eschar swabs. PCR buffy coat performed best with an overall positivity rate of 73% from day 1 to day 6, compared to ELISA/RDT. ELISA IgM and RDTs performed better from day 7 of fever onwards, with positivity rates of 90% and 81%, respectively. The combination of PCR buffy coat with an RDT detected 93% to 100% of all positive cases. PCR using eschar specimens yielded higher sensitivity when compared to PCR from buffy coat in paired matched samples.

Conclusions
Although PCR performance is superior during the first 7 days of fever and serology superior to PCR beyond this time point, the use of both modalities during the first 14 days of fever improves overall diagnosis. RDT shows great promise as a useful point-of-care test, particularly at community level and emergency departments.



Proportions of positivity diagnosed by PCR buffy coat, IgM ELISA, IgM RDT over time

The x-axis represents "Days of fever on admission" and "total confirmed case per day"; the first row states the days of fever reported on admission, and the second row states the total confirmed cases included per each respective day. The y-axis represents percentage.



Temporal dynamics of ELISA, RDT, PCR buffy coat and PCR eschar positive results in absolute numbers over time

Note: this graph includes eschar-positive cases. All cases with at least one or more modality testing positive using PCR eschar, PCR buffy coat and ELISA IgM among samples with all 3 tests available (n=151). RDT results are depicted as a dotted yellow line. X-axis represents "days of fever on admission", y-axis represents absolute patient numbers. The number of suspected cases seen per each day is represented by the blue bar (exact number stated above), of which the pale shaded blue bar represents the proportion of cases confirmed by PCR and/or ELISA by (single positive RDT results were not included as confirmed cases).

*Student paper

P-31 Profitability of pan-rickettsia real-time PCR for the molecular diagnosis of human rickettsioses in different clinical samples

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Background

Diagnosis of rickettsioses must be based on clinical and epidemiological criteria, although molecular techniques have allowed its confirmation quickly and reliably. As advantages, real-time PCR (qPCR) enables to quantify the PCR products while reducing time and costs when compared to conventional PCRs (cPCRs). Since several non-standardized PCR targets have been tested for *Rickettsia* detection in humans, our aim was to assess the usefulness (sensitivity) of a pan-rickettsia qPCR in clinical specimens for molecular diagnosis of rickettsioses.

Methods

495 samples (84 sera/81 whole EDTA-blood/78 plasma/77 erythrocyte layer/70 buffy coat/57 swabs/14 eschars collected at early phase of infection and, whenever possible, before antimicrobial therapy, and 34 detached ticks) from 144 patients diagnosed of acute rickettsioses were retrospectively selected (CRETAV collection: C.0006409).

Patients' inclusion criteria: clinical-epidemiological suspicion of rickettsiosis, with clinical samples subjected to cPCRs: *ompA/ompB*.

Pan-rickettsia probe-based *rplP*-qPCR was performed in all samples.

Results

Rickettsial infection was confirmed in 78/144 patients by *rplP*-qPCR *vs*. 71/144 by cPCRs.

Higher sensitivity was reached by *rplP*-qPCR *vs.* cPCRs in untreated (65,1% *vs.* 62,8%) and treated patients (45,8 % *vs.* 42,4%) (unknown data for n=42).

Rickettsia was detected in 156/495 samples using *rplP*-qPCR *vs*. 102/495 trough cPCRs.

Higher sensitivity was showed in blood and non-blood samples by *rplP*-qPCR *vs*. cPCRs (23,3% *vs*. 11,0% and 61,9% *vs*. 56,2%, respectively).

Higher sensitivity (equal in eschars) of *rplP*-qPCR *vs*. cPCRs in all types of samples.

The highest *rplP*-qPCR sensitivity was found in ticks, followed by swabs, eschars, buffy coat, plasma, serum, erythrocyte layer and whole EDTA-blood.

(Tables 1,2).

Conclusions

rplP-qPCR globally improves sensitivity compared to cPCRs for the molecular diagnosis of acute rickettsioses.

Buffy coat offered the best performance among blood samples.

Swabs (after ticks) are excellent *non-invasive* specimens for molecular diagnosis of rickettsioses, when available.

Table 1- Comparison of sensitivity of the 23S RNA real-time PCR (23S-qPCR) *vs.* conventional PCR (cPCR) assays for the molecular diagnosis of rickettsioses in patients included in this study.

	All patients					DEBONEL					MSF				
	N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs	
Total Sensitivity	144	76	52,8%	71	49,3%	71	37	52,1%	38	53,5%	55	24	43,6%	20	36,4%
Untreated	43	28	65,1%	27	62,8%	23	17	73,9%	17	73,9%	18	10	55,6%	9	50,0%
Treated	59	27	45,8%	25	42,4%	27	13	48,1%	13	48,1%	26	10	38,5%	8	30,8%
Sensitivity in blood samples	90	27	30,0%	18	20,0%	31	3	9,7%	3	9,7%	50	20	40,0%	14	28,0%
Untreated	23	10	43,5%	9	39,1%	4	0	0,0%	0	0,0%	18	10	55,6%	9	50,0%
Treated	38	10	26,3%	4	10,5%	10	0	0,0%	0	0,0%	24	8	33.3%	4	16,7%
Sensitivity in non-blood samples	86	61	70,9%	56	65,1%	58	38	65,5%	37	63,8%	14	9	64,3%	6	42,9%
Untreated	21	18	85,7%	18	85,7%	20	17	85,0%	17	85,0%					
Treated	38	23	60,5%	21	55,3%	25	13	52,0%	13	52,0%	9	6	66,7%	4	44,4%

	Rickettsia sibirica subsp. *mongolitimonae* infection					ATBF					Innominate rickettsioses[a]				
	N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs	
Total Sensitivity	11	10	90,9%	10	90,9%	4	2	50,0%	0	0,0%	3	3	100,0%	3	100,0%
Untreated	1	1	100,0%	1	100,0%	1	0	0,0%	0	0,0%					
Treated	3	2	66,7%	2	66,7%	1	0	0,0%	0	0,0%	2[b]	2	100,0%	2	100,0%
Sensitivity in blood samples	2	1	50,0%	1	50,0%	4	1	25,0%	0	0,0%	3	2	66,7%	0	0,0%
Untreated						1	0	0,0%	0	0,0%					
Treated	1	0	0,0%	0	0,0%	1	0	0,0%	0	0,0%	2[b]	2	100,0%	0	0,0%
Sensitivity in non-blood samples	10	10	100,0%	10	100,0%	1	1	100,0%	0	0,0%	3	3	100,0%	3	100,0%
Untreated	1	1	100,0%	1	100,0%										
Treated	2	2	100,0%	2	100,0%						2[b]	2	100,0%	2	100,0%

DEBONEL: *Dermacentor*-borne-necrosis-erythema-lymphadenopathy; MSF: Mediterranean spotted fever; ATBF: African tick-bite fever.
[a] Innominate rickettsioses include: 1 *Rickettsia aeschlimannii*, 1 *Rickettsia parkeri* and 1 *Rickettsia helvetica* infections.
[b] These two patients correspond to 1 *R. aeschlimannii* and 1 *R. parkeri* innominate rickettsioses.

Table 2- Comparison of sensitivity of 23S RNA real-time PCR (23S-qPCR) *vs.* conventional PCR (cPCR) assays according to the type of sample.

	All patients					DEBONEL					MSF				
Type of sample	N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs	
whole EDTA-blood	**81**	14	17,3%	6	7,4%	**25**	2	8,0%	2	8,0%	**50**	11	22,0%	4	8,0%
erythrocyte layer	**77**	15	19,5%	4	5,2%	**24**	2	8,3%	2	8,3%	**47**	12	25,5%	2	4,3%
serum	**84**	18	21,4%	10	11,9%	**28**	2	7,1%	2	7,1%	**48**	14	29,2%	8	16,7%
plasma	**78**	22	28,2%	16	20,5%	**24**	3	12,5%	2	8,3%	**47**	17	36,2%	13	27,7%
buffy coat	**70**	22	31,4%	7	10,0%	**22**	2	9,1%	1	4,5%	**48**	20	41,7%	6	12,5%
swabs	**57**	26	45,6%	21	36,8%	**35**	9	25,7%	8	22,9%	**12**	7	58,3%	4	33,3%
eschars	**14**	5	35,7%	5	35,7%	**10**	1	10,0%	1	10,0%	**0**				
ticks	**34**	34	100,0%	33	97,1%	**31**	31	100,0%	30	96,8%	**2**	2	100,0%	2	100,0%
Total	**495**	156	31,5%	102	20,6%	**199**	52	26,1%	48	24,1%	**254**	**83**	32,7%	**39**	15,4%
Blood samples	**390**	91	23,3%	43	11,0%	**123**	11	8,9%	9	7,3%	**240**	74	30,8%	33	13,8%
Non-blood samples	**105**	65	61,9%	59	56,2%	**76**	41	53,9%	39	51,3%	**14**	9	64,3%	6	42,9%

	Rickettsia sibirica subsp. *mongolitimonae* infection					ATBF					Innominate rickettsioses[a]				
Type of sample	N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs		N	23S-qPCR		cPCRs	
whole EDTA-blood	**1**	1	100,0%	0	0,0%	**2**	0	0,0%	0	0,0%	**3**	0	0,0%	0	0,0%
erythrocyte layer	**2**	0	0,0%	0	0,0%	**1**	0	0,0%	0	0,0%	**3**	1[b]	33,3%	0	0,0%
serum	**1**	1	100,0%	0	0,0%	**4**	0	0,0%	0	0,0%	**3**	1[c]	33,3%	0	0,0%
plasma	**2**	1	50,0%	1	50,0%	**2**	1	50,0%	0	0,0%	**3**	0	0,0%	0	0,0%
buffy coat	**0**					**0**					**0**				
swabs	**8**	8	100,0%	8	100,0%	**1**	1	100,0%	0	0,0%	1	1[c]	100,0%	1[c]	100,0%
eschars	**3**	3	100,0%	3	100,0%	**0**					1	1[b]	100,0%	1[b]	100,0%
ticks	**0**					**0**					1	1[d]	100,0%	1[d]	100,0%
Total	**17**	14	82,4%	12	70,6%	**10**	**2**	20,0%	**0**	0,0%	**15**	**5**	33,3%	**3**	20,0%
Blood samples	**6**	3	50%	1	16,7%	**9**	1	11,1%	0	0,0%	**12**	2[b,c]	16,7%	0	0,0%
Non-blood samples	**11**	11	100,0%	11	100,0%	**1**	1	100,0%	0	0,0%	**3**	3[b,c,d]	100,0%	3[b,c,d]	100,0%

DEBONEL: *Dermacentor*-borne-necrosis-erythema-lymphadenopathy; MSF: Mediterranean spotted fever; ATBF: African tick-bite fever.
[a] Innominate rickettsioses include: 1 *Rickettsia aeschlimannii*, 1 *Rickettsia parkeri* and 1 *Rickettsia helvetica* infections.
[b] The positive results in erythrocyte layer by 23S-qPCR and in a eschar by 23S-qPCR and cPCRs correspond to *R. aeschlimannii*.
[c] The positive results in serum by 23S-qPCR and in a swab by 23S-qPCR and cPCRs correspond to *R. parkeri*.
[d] The positive result in tick by 23S-qPCR and cPCRs correspond to *R. helvetica*.

P-32* **Cellular effects and target proteins of β-lactams and β-lactamase inhibitors in chlamydiae**

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[1] Institute for Pharmaceutical Microbiology, University of Bonn, University Hospital Bonn, Bonn, Germany, [2] Physical Chemistry, University of Bonn, Germany

Background

Chlamydiaceae have lost their cell wall in the course of evolution. Nevertheless, the genome-reduced bacteria build a transient peptidoglycan-ring to support cell division (Liechti *et al*., 2014). Unlike in other bacteria, treatment with β-lactams does not kill *Chlamydia*, but arrests cell division leading to reversible formation of persisting, non-dividing cells. *Chlamydia* harbor homologs of penicillin-binding proteins (PBPs) representing the primary target structures of β-lactams in most bacteria. Besides the two putative transpeptidases PBP2 and PBP3 and the carboxypeptidase PBP6 (Otten *et al*., 2015), our group identified LysM protein Cpn0902 of *C. pneumoniae* as a carboxypeptidase and cell division amidase AmiA as a novel target for penicillin (Klöckner *et al*., 2014).

Method

Please see below as integrated in the results section.

Results

In cell culture experiments, we found that not only β-lactams but also β-lactamase inhibitors like avibactam exhibit activity against *C. trachomatis* by inducing persistence. For some compounds like mecillinam and cefazolin, a transition state was observed with inclusions showing either the active or persistent chlamydial cell phenotype at the same inhibitory concentration. Complementary to conventional techniques, high resolution expansion microscopy was used to study chlamydial cells under penicillin treatment. We then analysed the affinity of various β-lactams and β-lactamase inhibitors for PBP6_Cp and PBP3_Ct *in vitro* using bocillinFL labeling assays. These experiments revealed differences in affinity for compounds like meropenem and imipenem in comparison to the respective *Escherichia coli* homologs and identified PBP3_Ct as a target of clavulanic acid. Furthermore, we addressed inhibition of the enzymatic function using peptidoglycan precursor lipid II as a substrate and found LysM protein Cpn0902 to be inhibited by penicillin.

Conclusions

Our research on β-lactam target proteins and other proteins of the chlamydial peptidoglycan-ring machinery will elucidate which enzymes and cellular mechanisms are targeted by β-lactams leading to arrested cell division and persistence in *Chlamydia*.

*Student paper

P-33 Eliciting protective CD8 T cell immunity to Chlamydia trachomatis infection using DNA vaccines

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Background

Chlamydia trachomatis is the most common cause of sexually transmitted bacterial infections worldwide and no vaccine is currently available for this pathogen. Major outer membrane protein (MOMP) based immunizations of mice have shown to elicit a robust humoral response, but they were not able to elicit protective CD8+ T cellbased immunity. Ideally, a protective *C. trachomatis* vaccine should elicit both humoral and cellular immune responses able to protect against intracellular bacterial challenge

Method

We used a murine model to test a DNA vaccination approach based on CTH522, a recombinant sequence including MOMPs of different *C. trachomatis* serovars, which was combined with an immune-stimulatory protein to increase immunogenicity. Immunizations took place on day 1, 4 and 7 and organs were collected on day 25. After restimulation with CTH522 derived epitope candidates, splenocytes were analyzed by intracellular cytokine staining and flow cytometry in order to quantify reactivated MOMP- specific CD8 T cells.

Results

This analysis led to the identification of a few immunodominant CD8 T cell epitopes.

Conclusions

Further studies will be carried out to extend the vaccine with high-affinity MHC class I binding epitopes predicted from the whole *C. trachomatis* proteome in order to guarantee a broader immune response, able to protect against bacterial challenges in vivo.

P-34* **Children throughout hotspots in Greece: a cross-sectional study**

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Background

Tropheryma whipplei is an intracellular bacterium considered to be transmitted from human to human via the oro-oral or fecal-oral route. Presence of *T. whipplei* seems to be increased among children and people living in poor conditions. The purpose of the study is to determine the presence of *T. whipplei* carriage in stool samples of migrant children and identify genotypes circulating among them.

Method

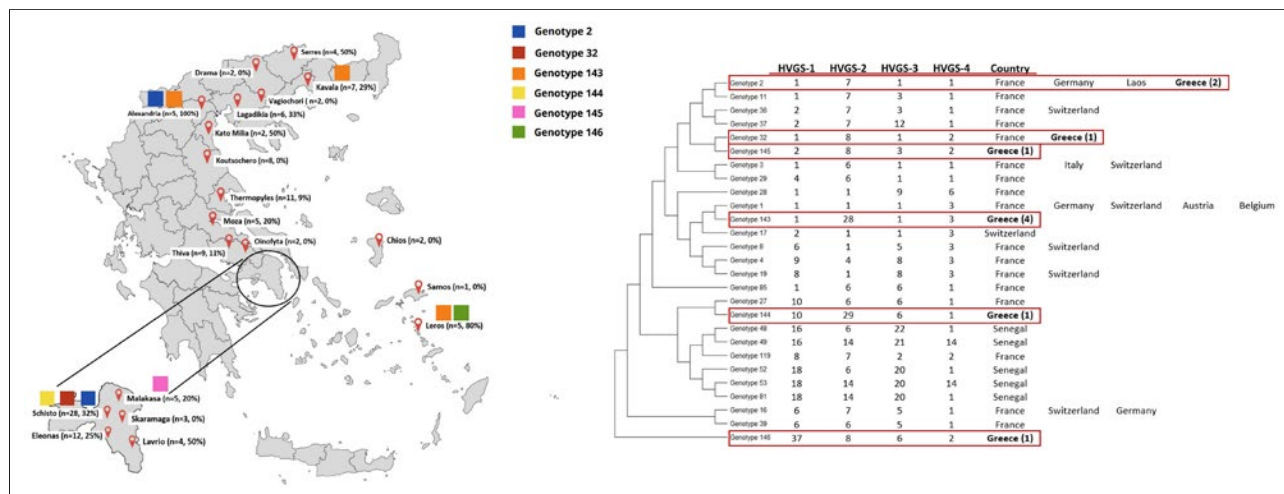
We tested stool specimens for *T. whipplei* obtained from children aged 0 to 12 years old living in 20 hotspots throughout Greece. All samples were screened using a quantitative PCR assay targeting two repeated sequences respectively. For positive samples, genotyping was performed targeting four highly variable genomic sequences (HVGSs).

Results

From 128 stool samples tested, 35 (27%) were positive for *T. whipplei* (54% males). Positive samples were found in 13 (65%) hotspots and the highest presence was observed in the Alexandria hotspot. Children aged 0-4 years (p=0.004) and particularly boys (p=0.06) have increased bacterial loads. Genotyping was achieved for 10 samples and six unique genotypes were defined, including four newly described. Genotype 143 and 2 were the most common ones. In addition, two children from the same family exhibited the same genotype reinforcing the fact of intrafamiliar circulation of *T. whipplei* strains.

Conclusions

We provide evidence of high presence of *T. whipplei* in fecal samples of children throughout the most hotspots in Greece. As *T. whipplei* is associated with acute diarrhoea in children, we emphasize the need of its systematical surveillance in migrants.



	HVGS-1	HVGS-2	HVGS-3	HVGS-4	Country				
Genotype 2	1	7	1	1	France	Germany	Laos	**Greece (2)**	
Genotype 11	1	7	3	1	France				
Genotype 38	2	7	3	1	France	Switzerland			
Genotype 37	2	7	12	1	France				
Genotype 32	1	8	1	2	France	**Greece (1)**			
Genotype 145	2	8	3	2	**Greece (1)**				
Genotype 3	1	6	1	1	France	Italy	Switzerland		
Genotype 29	4	6	1	1	France				
Genotype 28	1	1	9	6	France				
Genotype 1	1	1	1	3	France	Germany	Switzerland	Austria	Belgium
Genotype 143	1	28	1	3	**Greece (4)**				
Genotype 17	2	1	1	3	Switzerland				
Genotype 8	6	1	5	3	France	Switzerland			
Genotype 4	9	4	8	3	France				
Genotype 19	8	1	8	3	France	Switzerland			
Genotype 85	1	6	6	1	France				
Genotype 27	10	6	6	1	France				
Genotype 144	10	29	6	1	**Greece (1)**				
Genotype 48	16	6	22	1	Senegal				
Genotype 49	16	14	21	14	Senegal				
Genotype 119	8	7	2	2	France				
Genotype 52	18	6	20	1	Senegal				
Genotype 53	18	14	20	14	Senegal				
Genotype 81	18	14	20	1	Senegal				
Genotype 16	6	7	5	1	France	Switzerland	Germany		
Genotype 39	6	6	5	1	France				
Genotype 146	37	8	6	2	**Greece (1)**				

*Student paper

P-35 No Influence of Previous Coxiella burnetii Infection on ICU Admission and Mortality in Emergency Department Patients Infected with SARS-CoV-2

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Background

The geographical similarities of the Dutch 2007-2010 Q fever outbreak and the start of the 2020 coronavirus disease 19 (COVID-19) outbreak in the Netherlands raised questions and provided a unique opportunity to study an association between *Coxiella burnetii* infection and the outcome following SARS-CoV-2 infection.

Method

We performed a retrospective cohort study in two Dutch hospitals. We assessed evidence of previous *C. burnetii* infection in COVID-19 patients diagnosed at the ED during the first COVID-19 wave and compared a combined outcome of in-hospital mortality and intensive care unit (ICU) admission using adjusted odds ratios (OR).

Results

In total, 629 patients were included with a mean age of 68.0 years. Evidence of previous *C. burnetii* infection was found in 117 patients (18.6%). The combined primary outcome occurred in 40.2% and 40.4% of patients with and without evidence of previous *C. burnetii* infection respectively (adjusted OR of 0.926 (95% CI 0.605-1.416)). The adjusted OR of the secondary outcomes in-hospital mortality, ICU-admission and regular ward admission did not show an association either.

Conclusions

No influence of previous *C. burnetii* infection on the risk of ICU admission and/or mortality for patients with COVID-19 presenting at the ED was observed.

P-36 qPCR- and genomic-based determination of IS1111 copy numbers suggests an impact of Coxiella burnetii genotypic diversity on Q fever diagnosis and epidemiological studies in ruminants

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Background

The diagnosis of Q fever abortions in ruminants is routinely based on a qPCR assay targeting the multicopy IS1111 element. Here, we investigated whether phylogenetic relationships exist between IS1111 copy numbers in the genome and the diversity of *C.burnetii* genotypes, as this might impact the limit of detection and quantification of the method, and therefore be a source of epidemiological bias.

Method

We estimated IS1111 copy numbers using qPCR and *in silico* approaches, respectively: (1) considering 314 field samples from aborting females of known MLVA genotypes, TaqMan-based qPCR assays were used to quantify both the multicopy IS1111 and the monocopy *icd* targets in each sample, using plasmid calibration standards containing either of the targets; (2) considering full-genome sequences of 26 *C. burnetii* strains, newly obtained from aborting ruminants or available from GenBank, we calculated the ratio of the median (or mean) coverages obtained for IS1111 *versus* full genome sequences (minus IS1111 sequences), or we directly counted IS1111 sequences from blast outputs, respectively. We then explored, based on phylogenetic analyses, the relationships between the genotypic diversity and the number of IS1111 copies.

Results

Most considered samples and strains belonged to the main *C. burnetii* lineages circulating in Europe. Overall, IS1111 copy numbers varied between 12 and 228. The median and mean copy numbers were correlated with both the bacterial genomic group and, to a lesser extent, the ruminant host species.

Conclusions

We took up the challenge of combining both wet lab and *in silico* data in order to count IS1111 copy numbers in a broad panel of samples and isolates. We reported a clear variation among *C. burnetii* genomic groups, therefore confirming that strain diversity impacts the quantification and the sensitivity of bacterial detection, with potential consequences on Q fever diagnosis and epidemiological studies.

P-37 Ultraviolet C inactivation of Coxiella burnetii for production of a structural preserved whole cell antigen

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Background

Q fever, caused by *Coxiella burnetii*, is a worldwide-distributed zoonotic disease affecting humans and animals. Infections can cause economic losses and use of vaccines is limited.

Method

Inactivation of *C. burnetii* with ultraviolet light C (UVC, 100-280 nm) was evaluated for generation of a whole cell antigen with preserved bacterial cell structures and antigenic properties. Therefore *C. burnetti* Nine Mile phase I (NMI) and phase II (NMII) were exposed to decreasing intensities in a time-dependent manner and viability tested by rescue cultivation in axenic medium. Effects on the cell structure were visualized by transmission electron microscopy (TEM) and antigenic properties of UVC-treated NMI bacteria were studied by immunization of SPF-rabbits and antibody titration.

Results

NMI and NMII were inactivated at UVC intensities of 250 µW/cm2 for 5 min or 100 µW/cm2 for 20 min. Minor morphological changes, condensation and clumping of chromatin, dilatation of the periplasmic space, indicated shrinkage and was more pronounced in NMII than in NMI. UVC inactivated bacteria resulted in a pronounced antibody response in SPF rabbits of 1:128000 after the second booster. Applicability of UVC for inactivation of potentially infectious diagnostic samples was not successful. UVC treatment of field sera with known Q fever status and NMI-inoculated Q fever sera decreased antibody reactivity without complete bacterial inactivation.

Conclusions

Overall, UVC treatment inactivates *C. burnetii* cultured in axenic medium while preserving its cell structure and antigenic properties. The resulting product might be useful as a safe antigen for diagnostic purposes or as vaccine component.

P-38 Inferring clinical fluoroquinolone resistance from non-wild type parC genotype in Mycoplasma genitalium: mind the gap

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Background

Mycoplasma genitalium (MG) is a known agent of sexually transmitted infections. Treatment options are essentially azithromycin and moxifloxacin. High rates of macrolide resistance make timely diagnosis and monitoring of fluoroquinolone (FQ) resistance important. While resistance to quinolones has been linked to several mutations in the quinolone resistance-determining region (QRDR) of the topoisomerase gene *par*C, the relative contribution of each of these mutations to overall treatment failure is still being determined.

Method

A total of 636 individual MG-positive samples were collected between 2018 and 2021 and tested for macrolide and FQ resistance using real-time PCR. SNPs in the *23S rRNA* gene and amino acid (AA) changes in *parC* were analysed using the "LightMix Modular Assay" (TibMolbiol, Germany). Macrolide and FQ resistance is associated with mutations in the *23S rRNA* gene at positions 2058 and 2059 and with the following AA changes in the *parC* gene, respectively: S83I, S83R, D87N and D87Y.

Results

Of the 636 samples, 49 (7.7%) and 38 (6%) were undetectable by the 23S rRNA and parC-TibMolbiol assays, respectively. The prevalence of macrolide resistance was 34% (198/587), and FQ resistance was 23% (138/598). Both macrolide and FQ resistance were present in 12% (68/569) of the samples. The mutations associated with AA substitution were distributed in the *parC* gene as follows (see Fig.): S83I (n=54), S83R (n=7), D87N (n=8) and D87Y (n=4); mutations of unknown significance: S83N (n=21), G81C (n=1) and S84P (n=2). Additional 41 samples with AA changes in the QRDR must be clarified.

Conclusions

Resistance rates were high for macrolide and FQ. The published data on inferring FQ resistance from the *parC* genotype of MG are currently far from comprehensive. Consequently, culturing MG strains with QRDR mutations will be essential to reduce the knowledge gap between genotype and phenotype by quantitatively studying the phenotype of these isolates.

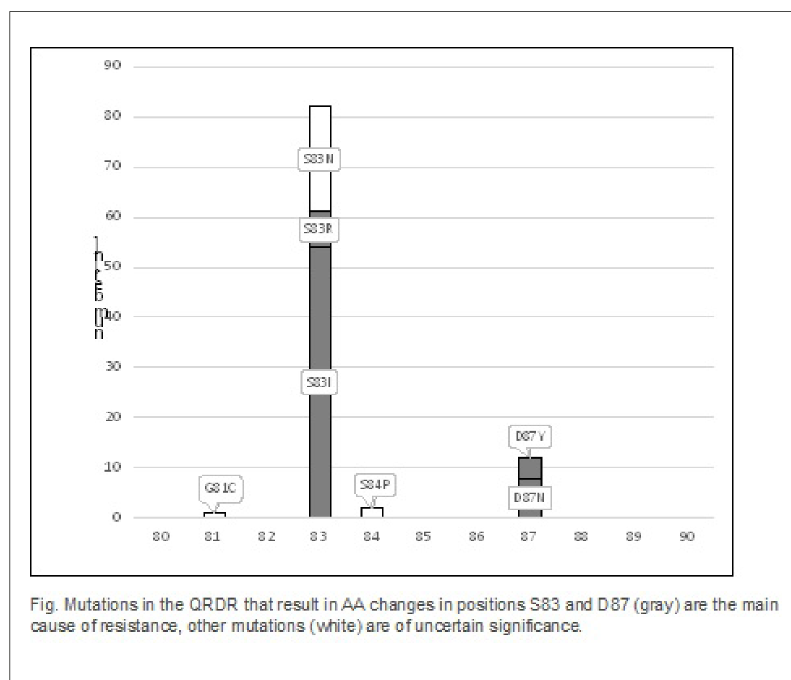

Fig. Mutations in the QRDR that result in AA changes in positions S83 and D87 (gray) are the main cause of resistance, other mutations (white) are of uncertain significance.

P-39 First confirmed case of 'Candidatus Neoehrlichia mikurensis' infection in a patient with antecedent of hematological neoplasm in Spain

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Background

'*Candidatus* Neoehrlichia mikurensis' is an α1-Proteobacterium (family *Anaplasmataceae*) transmitted by *Ixodes* ticks. In 2010, it was involved as human pathogen in Sweden. In Spain, it was described in *Ixodes ricinus* from cows in 2013, but it had not been detected causing human disease.

Method

Clinical case: '*Ca*. N. mikurensis' infection in an immunocompromised patient from Asturias (northern Spain) is described.

Results

A 68-year-old woman with antecedent of germinal centre diffuse large B-cell lymphoma (stage IV-B) diagnosed in a splenectomy piece who completed first-line treatment (R-CHOP) with complete response, begun with arthromyalgia/anorexia/night sweats/evening fever five months after finishing treatment. Few days later, she developed deep thrombophlebitis in her right leg. A relapsed lymphoma was suspected but a PET-CT scan showed diffuse and homogeneous bone marrow hyper-metabolism without data of tumor activity. Piperacillin/tazobactam and granulocyte-colony-stimulating factor were administered because neutropenia (400 neutrophils/mm3) was observed. Although the patient recovered from the neutropenia (attributed to methamizole intake to control their symptoms), fever persisted. Bone-marrow biopsy did not show tumor infiltration nor hematopoietic alterations. Hematology Service at Jarrio's Hospital (Asturias) contacted to CRETAV (La Rioja, Spain). Bone marrow DNA extract, sera (acute/4th and 6th-month) and EDTA-blood (4th and 6th-month) were sent for '*Ca*. N. mikurensis' PCR screening. Amplicons were detected in bone marrow, acute serum and EDTA-blood at 4th-month. Sequences corresponded to '*Ca*. N. mikurensis' (CP054597/MN701626) (Table 1; Figure 1). Re-interrogated, the patient remembered a tick-bite 20 days before the symptoms onset. She was treated with 100 mg doxycycline twice/day for three weeks. Fever disappeared at 72h.

Conclusions

1. This is the first documented and confirmed case of '*Ca*. N. mikurensis' infection from Spain.
2. '*Ca*. N. mikurensis' infection must be considered as a cause of persistent fever and venous thrombotic events in hematological malign disorder/immunosuppressed patients with possibility of tick-bite that do not respond to conventional therapy.

Table 1.- Results of sequence analyses obtained with different PCR targets for detection of '*Candidatus* Neoehlichia mikurensis'.

Stage of the disease	Clinical samples	PCR target genes The highest % identity (identical bp/total bp) GenBank accession number		
		Panbacterial 16S rRNA	16S rRNA-EHR	'*Ca*. N. mikurensis' *groEL*
Acute samples	Bone marrow	98% (1335/1362 bp) CP054597	100% (306/306 bp) CP054597	99.3% (1224/1233 bp) MN701626 *98.9% (1218/1232 bp) CP054597
	Serum	95.4% (753/789 bp)† CP054597	N.D.	99.3% (1224/1233 bp) MN701626 *98.9% (1218/1232 bp) CP054597
Samples after 4 months	EDTA-blood	98% (1305/1332 bp) CP054597	N.D.	N.D.
	Serum	N.D.	N.D.	N.D.
Samples after 6 months	EDTA-blood	N.D.	N.D.	N.D.
	Serum	N.D.	N.D.	N.D.

bp: base pairs; N.D. Not detected.
*For the *groEL* fragment gene, the % identity with the reference genome of '*Ca*. N. mikurensis' (GenBank accession number: CP054597), detected in a Swedish patient, was lower than with '*Ca*. N. mikurensis' isolate Omsk-41_Micagr, which was detected in a small mammal from Russian Siberia (GenBank accession number: MN701626).
† Sequence only obtained in one sense (insufficient sample).

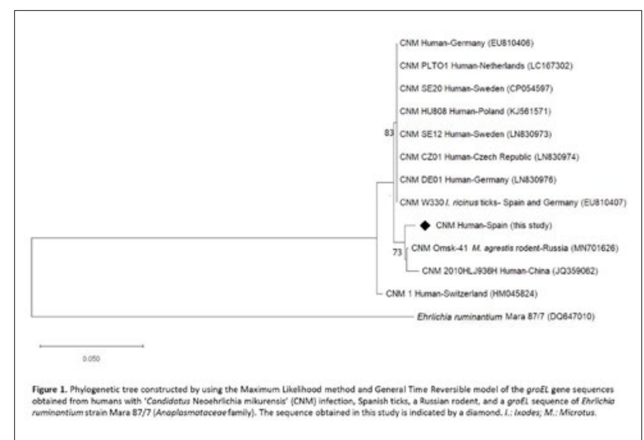

Figure 1. Phylogenetic tree constructed by using the Maximum Likelihood method and General Time Reversible model of the *groEL* gene sequences obtained from humans with '*Candidatus* Neoehrlichia mikurensis' (CNM) infection, Spanish ticks, a Russian rodent, and a *groEL* sequence of *Ehrlichia ruminantium* strain Mara 87/7 (*Anaplasmataceae* family). The sequence obtained in this study is indicated by a diamond. *I.*: *Ixodes*; *M.*: *Microtus*.

P-40 Molecular Detection of Rickettsia Using Quantitative Real-Time PCR in Fleas from South and East Georgia

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Background

Rickettsial pathogens cause diverse types of diseases in humans that vary in severity and clinical presentation. Some rickettsial pathogens include R. prowazekii, the causative agent of epidemic typhus, are Category B Select Agents. The flea-borne rickettsioses murine typhus and flea-borne spotted fever are febrile diseases distributed among humans worldwide, though there are no reliable epidemiological data for flea-borne Rickettsioses in Georgia. The main goal of the study was to estimate the prevalence of pathogenic species of Rickettsiae among fleas in the country. For this purpose we surveyed fleas collected in three regions of South and East Georgia.

Method

Total of 254 (n=4247 individual vectors) pooled flea samples were utilized for the study. Samples were collected under two different research protocols. The fleas were identified by team of entomologists and sample information was recorded. DNA was extracted using QIAamp Viral RNA mini kit. The genus–specific qPCR assay was used to detect the 17-kDa protein gene of Rickettsia species. Distribution and prevalence of rickettsiae in fleas was calculated as the minimum infection rate (MIR).

Results

The data from two different research protocols were analyzed separately. From the first protocol 135 (n=274) pools from East Georgia were tested and 17% (n=23) were positive. MIR for this region was 8.4. Result from another protocol: 119 (n=3973) pooled fleas from South Georgia, 4.2% (n=5) positives, MIR for South Georgia was calculated as 0.13.

Conclusions

For the first time in Georgia flea-born rickettsia was detected in samples collected in the country. Flea-borne rickettsial pathogens cause febrile diseases in humans worldwide, however, epidemiology and the public health risks these pathogens poses remains neglected in Georgia. This study provided valuable information on the prevalence and distribution of rickettsia in fleas in Georgia. This information will help to prevent or reduce the risk of rickettsial diseases exposure in newly identified

P-41 A history of Chlamydiae infection in Rheumatoid Arthritis development

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Background

Rheumatoid Arthritis (RA) pathogenesis is only partially understood. One hypothesis postulates an infectious etiology starting at the mucosal level. We investigated the association between *Chlamydiae* infection and the development of autoimmunity and pre-clinical manifestations associated with RA.

Method

This is a prospective cohort study of individuals genetically at risk of developing RA, namely first-degree relatives of RA patients (RA-FDR). We included all RA-FDRs who answered questions on *Chlamydiae* infections (self-reported history). In a nested case-control study, we also explored the serological status against *Chlamydia trachomatis*. The primary outcome was RA-associated autoimmunity (RA-autoimmunity) defined by the presence of anti-citrullinated protein antibodies and/or rheumatoid factor at the last visit; secondary outcomes were 1. High levels of RA-autoimmunity (levels> 3x upper limit of the norm); 2. RA-associated symptoms with RA-autoimmunity; 3. Subsequent diagnosis of seropositive RA.

Results

Among 1231 RA-FDRs analyzed, 168 (13.6%) had developed RA-autoimmunity. The prevalence of self-reported *Chlamydiae* infection was significantly higher in individuals with RA-autoimmunity as compared to controls (17.9% versus 9.8%, P<0.01). A significant association between the self-reported history of *Chlamydiae* infection and RA-autoimmunity was observed in both univariate and multivariate analyses (OR=2.00, 95%CI: 1.27-3.09; OR=1.91, 95%CI: 1.20-2.95, respectively). Similar associations were also found in later stages of pre-clinical RA (secondary outcomes, Figure 1A). In addition, we found a similar trend when analysing *C.trachomatis* serology in a nested case-control study (n=186 seropositive RA-FDRs cases; n = 186 asymptomatic and seronegative RA-FDRs controls), with a significant association between anti-MOMP IgG antibodies and high levels of RAautoimmunity, but weaker associations in other pre-clinical stages of RA.

Conclusions

Our results suggest that a history of *Chlamydiae* infection may be a risk factor for subsequent development of RA in a subset of individuals genetically at risk for the disease.

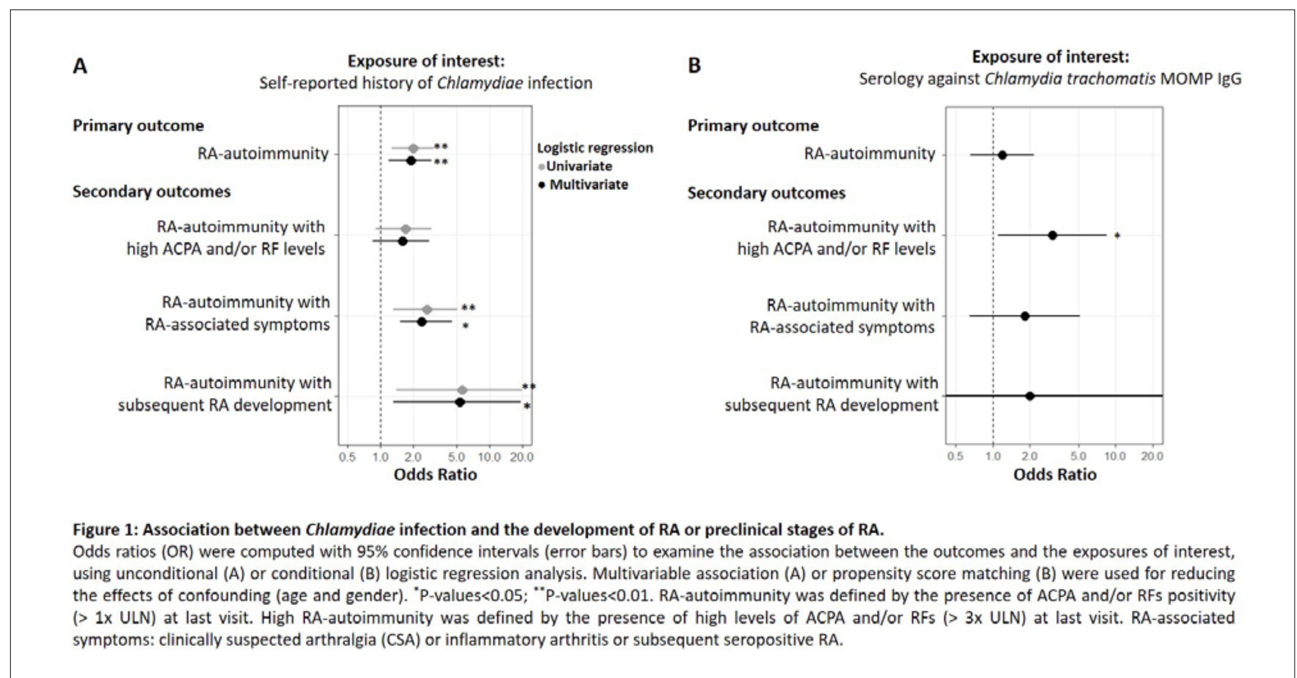

Figure 1: Association between *Chlamydiae* infection and the development of RA or preclinical stages of RA.
Odds ratios (OR) were computed with 95% confidence intervals (error bars) to examine the association between the outcomes and the exposures of interest, using unconditional (A) or conditional (B) logistic regression analysis. Multivariable association (A) or propensity score matching (B) were used for reducing the effects of confounding (age and gender). *P-values<0.05; **P-values<0.01. RA-autoimmunity was defined by the presence of ACPA and/or RFs positivity (> 1x ULN) at last visit. High RA-autoimmunity was defined by the presence of high levels of ACPA and/or RFs (> 3x ULN) at last visit. RA-associated symptoms: clinically suspected arthralgia (CSA) or inflammatory arthritis or subsequent seropositive RA.

P-42 **Multilocus Sequence Based Phylogenetic Analysis of Candidatus Rickettsia Barbariae in Georgia**

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Background

Candidatus Rickettsia barbariae a member of the spotted fever group rickettsia (SFGR), is an incompletely described agent that has yet to be fully characterized. Previous study revealed presence of Candidatus R. barbariae DNA among Rickettsia positive tick pools in Georgia (9.4%). In this study phylogenetic analysis of three rickettsial genes (*gltA*, *ompA* and *ompB*) on C. *Rickettsia barbariae* positive DNAs were conducted for more clear characterization of this SFGR and to confirm the phylogenetic relationship between Georgian strains and other rickettsiae.

Method

Multilocus sequence typing (MLST) using partial sequences of citrate synthase *gltA* and outer membrane proteins - *ompA* and *ompB* genes were conducted on eight DNA samples, identified as *C.R.barbariae* using ABI Prism 3130xl Analyzer. Phylogenetic analyses were performed based on the multialignment of *gltA*, *ompA* and *ompB* sequences of Georgian samples and other rickettsial isolates from GenBank.

Results

The phylogenetic analysis based on partial sequences of gltA, ompA, ompB genes clustered Georgian strains among C.R.barbariae group. gltA sequences didn't reveal deferences among Georgian strains. OmpA sequence based analyses identified two clusters; Georgian strains formed one cluster with R. barbariae strain from Algeria, Lebanon , "India-Sardinia", china (KU645284.1). BLAST analysis of partial cds of *ompB* gene (1432bp) of all eight Georgian C. *R. barbariae* showed 100% similarity with a homologous fragment of strain EU272187.1 -"India: Sardinia" and in the phylogeny tree they form one sub-cluster.

Conclusions

High prevalence and wide distribution of Rickettsia species among ticks makes rickettsiosis a potential public health problem in Georgia. Three partial genes sequence based phylogenetic analysis of *C.R.barbariae* obtained from three different tick species collected from different hosts in various regions of Georgia did not reveal the difference among strains, suggesting a single introduction of the C. *R. barbariae* in Georgia. Further studies including other gene sequences could provide more profound understanding of phylogeny of C.*R.barbariae*.

P-43* Revealing the hidden protective potential of selected cell-autonomous defense programs in the battle against Chlamydia trachomatis

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Background

Causing more than 100 million cases of urogenital infections every year, *Chlamydia trachomatis* is among the most prevalent agents of sexually transmitted diseases. As common for successful intracellular pathogens, *C. trachomatis* has evolved virulence strategies to evade the powerful defense programs of its host cell. However, our understanding of their significance and of the underlying molecular processes is still scarce.

Method

To address this knowledge gap, we used the CRISPR/Cas9 approach to disable selected defense programs in cultured cells, allowing us to assess their relevance in restricting the intracellular growth of *C. trachomatis* and the growth of its non-pathogenic relatives. Moreover, the generation of these cells enabled a forward genetic screen of a *C. trachomatis* mutant library, being conducted to identify mutants that have a survival or growth benefit in cells with disabled defenses.

Results

Up to now, we specifically addressed the roles of xenophagy, necroptosis, and type I interferon driven responses. We observed that wild-type *C. trachomatis* did not apparently benefit from disabling these defenses. Experiments with non-pathogenic *Chlamydia*-like bacteria are ongoing. The screening of the *Chlamydia* mutant library will be completed by June 2022 and hits will then be followed up upon.

Conclusions

The seemingly undisturbed growth of wild-type *C. trachomatis* in defense-deficient host cells is in line with our assumption that the pathogen is well adapted to its intracellular niche and has found effective ways to neutralize the defenses of its host cell. We expect that the ongoing mutant library screen will provide a better understanding of which defense programs could indeed be protective, if not suppressed by the pathogen. Moreover, it will enable an identification of the suppressing virulence factors. Together, this knowledge may in the future inspire novel treatment strategies.

*Student paper

P-44 **Infection of the nematode Caenorhabditis elegans with Chlamydia pneumoniae and Chlamydia trachomatis**

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Background

Despite the role of chlamydial persistence in driving chronic infections, the host – pathogen relationships in this context remain elusive. Bridging the translational gap between *in vitro* cell culture methodologies and *in vivo* experimentation requires novel approaches, one of which is represented by *Caenorhabditis elegans*, a nematode with just 1000 cells but harboring innate immunity signaling pathways conserved also in higher organisms.

Method

Adult *C. elegans* worms were inoculated with *C. pneumoniae* strain CV6 or *C. trachomatis* serovar K in liquid medium and subsequently transferred onto standard nematode culture conditions on agar plates. The lifespan of the worms was followed over the course of 25 days and on days 1, 3 and 6 worms were lysed and total DNA extracted for quantitative PCR analysis applying primers for *C. pneumoniae ompA* gene.

Results

Consistent with earlier observations with other pathogenic bacteria, both *C. pneumoniae* and *C. trachomatis* decreased the time-adjusted survival rate of the worms and shortened the worm lifespan (***$p<0.001$ in both cases). Treatment of the worms with bleach followed by incubation with DNAse was found a suitable procedure for the removal of noninternalized *Chlamydia* elementary bodies prior to DNA extraction. The qPCR data confirmed the chlamydial entry into nematode cells. The genome copy numbers remained constant over the period of 6 days post infection, indicating bacterial persistence rather than active replication inside the host.

Conclusions

This proof of concept study demonstrates that *C. elegans* can be infected with the human pathogens *C. pneumoniae* and *C. trachomatis*. Owing to the immense genetic toolkit available for *C. elegans,* this ethically and economically sustainable *in vivo* infection model can be expected to significantly contribute to host - *Chlamydia* interaction studies in the future.

P-45 The development of the 1st International standard for Q fever based on anti-Coxiella burnetii plasma IgG (human)

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Background

Q Fever is a worldwide zoonotic disease caused by the gram-negative coccobacillus Coxiella burnetii. C. burnetii is highly infective it is transmitted from animals to humans by inhalation of contaminated dust from sheep, goats and cattle. In about 50% of all cases, infection with C. burnetii causes either acute or chronic disease. Up to 20% of individuals with symptomatic infection develops Q fever fatigue syndrome with 1-5% progressing to persistent focalized infection e.g. in heart valves or large vessels (chronic Q fever). A large outbreak in the Netherlands between 2007-2010 resulted in 4000 notified cases and an estimate >40,0000 infections, emphasizing the fact that Q fever can become a major public health concern. Serological assays to assess the anti-C. burnetii antibody responses are an important tool in the diagnosis of chronic Q fever, surveillance and pre-screening for prior exposure. Currently available assays include the IFA, ELISA and CFA. Comparability of these assays are difficult since many laboratories use their own in-house assays. Fluorescence in the most sensitive assay, the IFA, can be highly subjective. A Q fever reference standard would aid quality control of assays between different laboratories, support development and validation of novel serological tests, as well as support comparative studies of the immunogenicity of novel Q fever vaccines in differing clinical trials.

Method

Hence we are developing the first International Q fever reference standard, prepared from a pool of plasma from C. burnetii sero-positive individuals. Two patients were selected for plasmapheresis based on phase 1 and phase 2 IgG levels. A trial fill of this potential Q fever reference standard will be prepared and characterised.

Results

N/A

Conclusions

Once proof of concept has been determined a definitive fill and a collaborative validation study will be implemented. The final standard will be available for clinical trials and laboratory studies in early 2022.

P-46* **In situ architecture of intracellular bacterial communities in urinary tract infections**

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Background

Urinary tract infections (UTIs) are among the most common bacterial infections. 80% of UTIs are caused by uropathogenic *Escherichia coli* (UPEC). Even though UTIs can be treated with antibiotics, 25% of patients suffer from a recurrent UTI within six months, which is often caused by the same bacterial strain as the initial infection. UPEC can proliferate in the bladder lumen, but they can also invade and replicate within superficial umbrella cells to form intracellular bacterial communities (IBCs). These IBCs display biofilm-like properties and protect the pathogens from host defenses and antibiotics, presumably contributing to recurrent UTIs. However, the native architecture of IBCs and the molecular processes underlying recurrent infections still remained elusive.

Method

To analyze IBC formation from molecular to cellular scale, we optimized an UPEC infection protocol using a bladder cell line. UPEC were expressing a cytoplasmic fluorescent marker to follow the infection cycle by live cell imaging. The optimized infection protocol was transferred to cells seeded on electron microscopy (EM) grids. After vitrification by plunge-freezing, we used cryo-fluorescent light microscopy (cryo-fLM) to detect different maturation stages of IBCs. The cryo-fLM maps were used to correlate cryo-FIB milling for sample thinning and cryo-ET to reveal the native *in situ* architecture of IBCs.

Results

Simultaneous infection of the bladder cells with UPEC strains expressing either GFP or mCherry, resulted in the formation of mainly clonal IBCs. Our cryo-ET data revealed that IBCs are composed of densely packed bacteria, which are embedded in an extracellular matrix while expressing many surface structures, such as pili or flagella.

Conclusions

UPEC form clonal biofilm-like assemblies in infected bladder cells. The analysis of the acquired tomograms revealed the near-native architecture of IBCs. The observed UPEC surface structures within IBCs will serve as starting point to delete specific virulence factors to further validate their role during IBC formation.

*Student paper

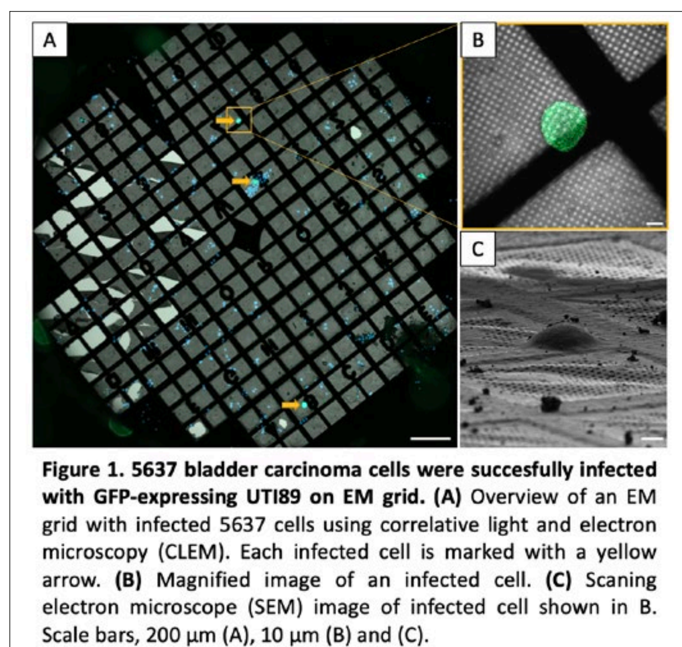

Figure 1. 5637 bladder carcinoma cells were succesfully infected with GFP-expressing UTI89 on EM grid. (A) Overview of an EM grid with infected 5637 cells using correlative light and electron microscopy (CLEM). Each infected cell is marked with a yellow arrow. **(B)** Magnified image of an infected cell. **(C)** Scaning electron microscope (SEM) image of infected cell shown in B. Scale bars, 200 µm (A), 10 µm (B) and (C).

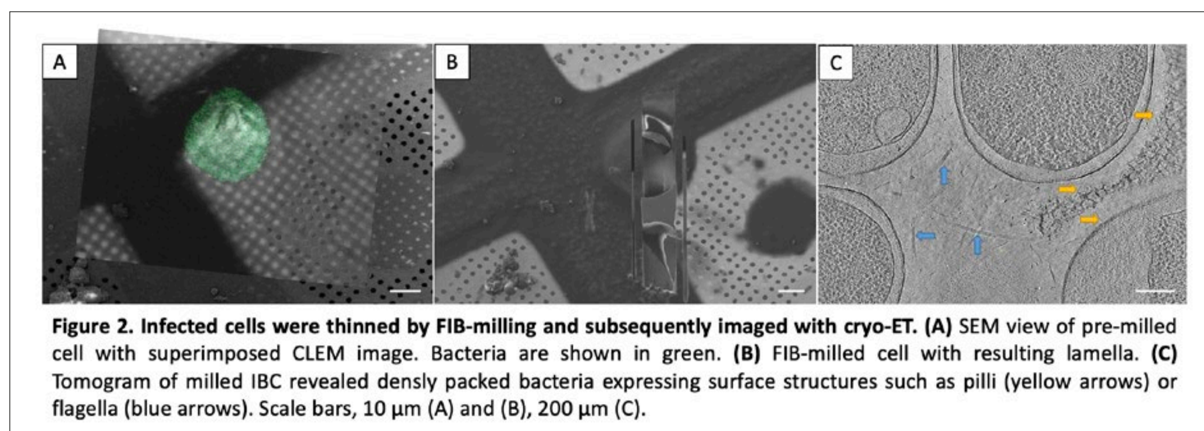

Figure 2. Infected cells were thinned by FIB-milling and subsequently imaged with cryo-ET. (A) SEM view of pre-milled cell with superimposed CLEM image. Bacteria are shown in green. **(B)** FIB-milled cell with resulting lamella. **(C)** Tomogram of milled IBC revealed densly packed bacteria expressing surface structures such as pilli (yellow arrows) or flagella (blue arrows). Scale bars, 10 µm (A) and (B), 200 µm (C).

P-47* Identification and Functional Analysis of Coxiella burnetii Dugway Strain-Specific Type IV Secretion System Effector Proteins

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Background

Coxellia burnetii is a gram-negative intercellular bacterium that is the causative agent of Q fever. Livestock are the primary natural reservoir of *C. burnetii* and majority of human infections are acquired by inhalation of contaminated aerosols derived from these animals. In 1957, at the Dugway Proving Grounds, Utah, USA, genetically unique strains of *C. burnetii* were isolated from wild rodents. Despite retaining a primary virulence factor, full-length lipopolysaccharide, these strains were reported to exhibit avirulence *in vivo*.

Method

We confirmed this observation in a guinea pig intraperitoneal infection model, with Dugway-infected animals displaying similar trends in body temperature and weight as that of saline mock infected animals, diverging from the positive, virulent infection control. Additionally, a Dugway Δ*dot/icm* strain was generated that lacked the type IV secretion system (T4SS) and infection with this strain resulted in a similar lack of virulence.

Results

Dugway strains display the largest *C. burnetii* genomes which include T4SS effector genes. *C. burnetii* T4SS effector proteins are important for several bacterial and host functions, including host cell survival and immune modulation. We hypothesize that these unique T4SS effector proteins may contribute to Dugway's avirulence via interaction with host cells. After confirming Dugway avirulence *in vivo*, we identified 23 Dugway-specific putative T4SS effector genes and confirmed bacterial secretion by an adenylate cyclase reporter assay. In order to determine the potential role(s) of these effector proteins in Dugway avirulence, we designed fluorescently tagged Dugway effector fusion proteins to be utilized in mammalian ectopic expression assays. Select effector proteins have been analyzed for ectopic expression and generally display non-specific localization patterns. Additional RNA sequencing analysis in infected human macrophage-like cells is underway.

Conclusions

This work will lead to improved functional understanding of Dugway strains and related T4SS proteins during infection and elucidation of important factors influencing *C. burnetii*-host interactions.

*Student paper

P-48 **Comparative analysis of the type IV secretion systems (T4SS) in eight complete genomes of Orientia tsutsugamushi**

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Background

Orientia tsutsugamushi (*Ot*) is an obligate intracellular bacterium that causes a serious health problem (scrub typhus) in the Asia-Pacific region. *Ot* has an unusually large and reshuffled genome compared with other Rickettsiaceae, driven by the presence of an integrative conjugative element called RAGE (Rickettsia Amplified Genetic Element) which has undergone rampant replication throughout the *Ot* genome. The RAGEs of *Ot* are present in 80-90 mostly degraded copies per genome and are poorly annotated.

Method

We aimed to bioinformatically analyse the entire genome of *Ot* and reannotate the uncharacterized RAGEs across 8 *Ot* strains (Boryong, Ikeda, UT76, UT176, Karp, Kato, Gilliam, and TA686) with a specific focus on T4SS.

Results

Our analysis led to the identification of complete RAGEs in some genomes of *Ot*. Two major T4SSs are present in *Ot* namely *vir* (ortholog of *Agrobacterium tumefaciens* P plasmid) and *tra/trb* (ortholog of *Escherichia coli* F plasmid). The analyses revealed the presence of all *vir* components except 2 (*VirB1, VirB5*) and show gene multiplication in *virB2, virB4*, *VirB6*, *VirB8*, and *VirB9* similar to other *Rickettsiales*. In F plasmid gene analyses, *Ot* retains as many as 18 *tra/trb* components out of the 28 *tra/trb* components in *E. coli*. The *tra/trb* components are encoded on the RAGEs and are present in high numbers although most gene copies are truncated pseudogenes. However, some *Ot* strains encode a complete set of full length *tra/trb* genes, suggesting they may still be able to form a competent transport apparatus to transport the RAGE. We analyzed proteomics expression data and found that only *vir* genes were translationally expressed under the laboratory growth conditions used.

Conclusions

This supports the functionality of *vir* in *Ot* and a degradation of *tra/trb*. This work will facilitate future studies by providing a foundation and a better understanding towards *Ot* obligate intracellular lifestyle and its pathogenicity.

P-49 Rickettsia cause deregulation of genes coding for the neurotoxic cell response pathways in cerebrocortical neurons cultured in vitro

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Background
Rickettsia can cross the blood-brain barrier and cause infection of central nervous system. The infection can result in several symptoms such as meningitis, encephalitis, acute disseminated encephalomyelitis, and others. Despite the considerable health danger, just a few studies on the pathogenesis induced by Rickettsia in CNS have been conducted. Moreover, in the context of etiopathogenesis of neurodegeneration, no detailed mechanisms were described so far, although in animal models their pro-apoptotic effect on neurons was demonstrated.

Method
In order to investigate the molecular signaling associated with neurotoxic effects of Rickettsia we employed an experimental model of rat embryonal neurons. Using a panel of 29 qPCR probes, we profiled the expression of key regulatory genes important in the neurotoxic cell response pathways after infection with *Rickettsia akari* and *Rickettsia slovaca*.

Results
We found a strong reduction in viability of rat cerebro-cortical neurons 36 hours after rickettsial infection in vitro, which was associated with the deregulation of several genes involved in pro-inflammatory signaling, regulation of apoptosis, and cell death.

Conclusions
In conclusion, our data further clarify the underlying molecular mechanisms involved in rickettsial infection of neuronal cells and identify key regulatory signaling pathways linked to the neurotoxic effects of Rickettsia species. Funding: This study was financially supported by the Slovak Research and Development Agencies: APVV-19- 0066 & APVV-17-0668, as well as VEGA: 2/0010/19 & 2/0153/22.

P-50 Parasitic infestation (Parasitengona, and the mite: Oribatida: Brachypylina Brachypyline Oribatid) new records in disease and zoonosis transmission by migratory birds Fulica atra and Anas platyrhynchos in Algeria

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Background

summary
In the wetland of Lake Tonga (El Tarf), in the northeast of Algeria, between March and May 2017, we collected ten nests of migratory birds, the coot coot Fulica atra and the mallard duck Anas platyrhynchos. Morphological identification of ectoparasites has shown a dominance of arthropods parasites, with (59.5%) mites, 6 Parasitengona mites oribatidae mites Brachypyline, Trombidiformes "velvet mites" considered as arthropods important from a medical point of view, can transmit parasitic diseases (such as typhus exfoliant), and 44.04% between arachnids, pseudoscorpions, beetles, nematodes and gastropods (molluscs).
Keywords: Fulica atra, Anas platyrhynchos, arthropods, moth, wetlands, Algeria.

Method

Morphological identification of ectoparasites is based on laboratory MARBREC. CNRS "Ecology of coastal marine systems (Ecosym)". UMR 5119, CNRS, IRD, Ifremer Place Eugene Bataillon Montpellier University, Case 093, F- 34095 Montpellier Cedex 5 France.

Results

Morphological identification of ectoparasites has shown a dominance of arthropods parasites, with (59.5%) mites, 6 Parasitengona mites oribatidae mites Brachypyline, Trombidiformes "velvet mites" considered as arthropods important from a medical point of view, can transmit parasitic diseases (such as typhus exfoliant), and 44.04% between arachnids, pseudoscorpions, beetles, nematodes and gastropods (molluscs).

Conclusions

Parasitengona mites, oribatidae mites, Brachypyline, and Trombidiformes "velvet mites" are considered as important arthropods from a medical and bacterial disease, can transmit zoonose diseases (such as typhus exfoliant)

P-51* **Characterization of Nucleotide Diphosphate Kinase in Chlamydiae**

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Background

Transition between the two developmental forms of *Chlamydiae*, the infectious Elementary Body (EB) and the replicating Reticulate Body (RB), is central for chlamydial replication and pathogenesis; nevertheless, the intrinsic molecular triggers of transition are unknown. There is growing evidence that Nucleoside Diphosphate Kinase (NDK) gene family plays a role in the differentiation of developmental forms in microorganisms such as *Myxococcus xanthus* and *Aspergilus flavus*. *Waddlia chondrophila*, a *Chlamydia*-related bacterium, carries two copies of NDK (designated as WcNDK1 and WcNDK2), which are organized in an operon. Interestingly, the Wc- NDK2 is fused to an N-terminal signal peptide. *Chlamydia trachomatis* carries only one copy of the gene (CtNDK). Here we aim to characterize the chlamydial NDK gene family and to study its significance in development of *Chlamydiae*.

Method

We performed RT-qPCR to investigate the NDK gene expression pattern throughout *C. trachomatis* and *W. chondrophila* developmental cycles. We expressed WcNDK1, WcNDK2 and CtNDK in Hela cells, to study their subcellular localization.

Results

NDK genes expression pattern is conserved in *W. chondrophila* and *C. trachomatis* and shows a peak around 8 hpi. WcNDK1 and CtNDK were detected in the cytoplasm of transfected Hela cells. Full-length WcNDK2 is translocated to the nucleus; however, its localization is cytoplasmic in the absence of signal peptide.

Conclusions

Conservation of the NDK gene expression pattern between the two *Chlamydiae* members might indicate involvement of these kinases in conserved processes such as development. In addition, this study suggests a role for WcNDK2 signal peptide as nuclear translocator. Identifying interaction partners of chlamydial NDKs by immunoprecipitation and determining DNA binding ability of WcNDK2 is currently ongoing. The role of NDK genes in development of *Chlamydiae* could be further investigated by applying NDK specific inhibitors and this could pave the way for drug development based on NDK-specific inhibitors.

*Student paper

P-52 Epidemiological investigation on a dairy sheep farm in a professional agricultural high school following an alert of Q fever clustered human cases

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Background

Between January and May 2019, twelve human cases of Q fever were suspected in an agricultural vocational school owning a dairy sheep farm, in France. Finally, a single human case was confirmed. Nevertheless, up to 300 people could be considered exposed (students, staff). In October 2018, Q fever had already been identified in a few aborted adult ewes; then, more than 40% of the primiparous ewes aborted between late December and February 2019. The sheepfold, located near the farm and school buildings, is surrounded by pastures. In addition, this herd summers annually in mountain pastures together with other herds. This context prompted public and veterinary health stakeholders to conduct investigations and to recommend management measures.

Method

Management strategies were implemented. Public visits and scholar activities related to the farm ceased from January 21st 2019. All sheeps including lambs were vaccinated. The sheepfold was cleared out and the manure piled up, sheltered from the wind and then composted. Two cleanings and disinfections (C/D) were carried out, the last one using a sporicide after animals' departure for summer. At each critical step, qPCR was performed on dust wipes sampled from various relevant sites and on vaginal swabs and wool sampled from ewes.

Results

At the time of the second wave of abortions, bacterial shedding by both aborting and non-aborting ewes and environmental contamination were massive. Then, the monitoring of bacterial loads revealed a decrease in animal shedding but a persistence of high environmental contamination. This persistence was confirmed the following year, with however a tendency to decrease while using the usual hygiene practices. The shedding level remained very low.

Conclusions

The effect of C/D appeared negligible on surface contamination estimated by qPCR. Overall, transmission to humans was low despite intense animal shedding and persistent environmental contamination.

P-53* The Neoferon study – a study to answer if Neoehrlichia mikurensis causes chronic persistent infections in human beings

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Background

Neoehrlichia (N.) mikurensis causes the infectious disease neoehrlichiosis in humans via tick bites. Fever and vascular events are typical findings. These intracellular bacteria do not grow in cell-free culture media and consequently escape detection by blood culture. The incubation period of neoehrlichiosis is uncertain. Several studies imply that long-term asymptomatic carriage of *N. mikurensis* can occur. Rituximab, a monoclonal antibody that targets CD20 on B cells, is a risk factor for severe neoehrlichiosis. The goal of the Neoferon study is to determine if *N. mikurensis* causes latent infections that can reactivate when B-cell defenses are suppressed.

Method

The Neoferon study was initiated in the summer of 2021 in collaboration with the Department of Hematology at the Sahlgrenska University Hospital in Sweden. Adult patients diagnosed with malignant B cell lymphomas are monitored before, midway and after termination of rituximab therapy for levels of *N. mikurensis* DNA in the blood by specific Real Time PCR.

Results

A total of 49 patients have been recruited to the study so far. Four patients (8.2 %) tested positive for *N. mikurensis* already before the start of rituximab treatment, and two patients (4.1 %) tested positive for *N. mikurensis* midway (n = 1) and after termination (n = 1) of the rituximab treatment period. None of the patients had fever or any other symptoms of infection.

Conclusions

An unexpectedly high percentage of patients with malignant B cell lymphoma appeared to be asymptomatic carriers of the infection prior to the start of B cell suppressive therapy with rituximab. These findings support the hypothesis of latent *N. mikurensis* infections in lymphoma patients, even before start of immunosuppressive therapy.

*Student paper

P-54 High risk of ruptured aneurysm after vascular infection with Coxiella burnetii: clinical outcomes in patients with vascular chronic Q fever

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Background
Infection of aortic or iliac aneurysms is a rare but life-threatening disease. After a large Q fever outbreak in the Netherlands, vascular chronic Q fever (CQF), chronic infection of an aneurysm or vascular prosthesis with *Coxiella burnetii,* was often observed. A large proportion of patients with vascular CQF died due to complications. We provide an overview of characteristics and clinical outcomes of vascular CQF patients with an additional focus on the growth pattern and rupture risk of aortic and iliac aneurysms in these patients.

Method
Data were retrieved from the Dutch National Chronic Q Fever Database, containing data from CQF patients diagnosed during and in the 10 years after the large Q fever outbreak in the Netherlands.

Results
We identified a total of 282 vascular CQF patients, with a median follow-up time of 47.5 months (interquartile range 17-92 months). Q fever-related mortality was 30.1% (n=85), and 12.7% of patients (n=36) died directly due to complications of their infected aneurysm. A ruptured aneurysm occurred in 14.5% of all vascular CQF patients, and it was the first presentation of an aneurysm in 7.1% of the patients. In the 21 patients presenting with a ruptured aneurysm as first presentation, the aneurysm did not reach the cut-off diameter for surgery in 28.6%. Furthermore, 5 of the 6 CQF patients who at imaging of their aneurysm in the 6 or 12 months before rupture, had their ruptured aneurysm in the watchful waiting interval advised in the guideline.

Conclusions
Vascular CQF has high morbidity and disease-related mortality. Aneurysms infected with *C. burnetii* seem to have an aberrant and unpredictable growth pattern with a high early rupture chance. Therefore, treating physicians should consider to perform early aneurysm repair in vascular CQF patients.

P-55 Does environmental water support the survival of Francisella tularensis, the etiological agent of tularemia?

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Background

Tularemia is a zoonosis caused by the intracellular facultative bacterium *Francisella tularensis*. Human contamination mainly occurs through contact with wildlife animals or arthropod bites. Tularemia can also be a water-borne disease. However, *F. tularensis* mechanisms of survival in aquatic environments are poorly characterized.

Method

Firstly, using *in vitro* models, we investigated interactions of *F. tularensis* subsp. *holarctica* (the tularemia agent found in the northern hemisphere), and the aquatic species *F. novicida* and *F. philomiragia* with amoebae of the *Acanthamoeba* species. Secondly, we conducted an environmental study by collecting surface water samples in a tularemia endemic region in France. We investigated the presence of *F. tularensis* and other *Francisella* species in water using a set of qPCRs.

Results

In amoeba plate screening tests, all the *Francisella* species tested resisted the attack by amoebae. In infection model, focusing on strictly intra-amoebic growth, none of the *Francisella* species tested displayed any intraamoebic multiplication. In co-culture models, focusing both on intra and extra-amoebic bacterial multiplication, the amoebae favoured *Francisella* survival over 16 days (Figure 1) and preserved bacterial morphology (Figure 2). Two sampling campaigns were conducted in West of France, in July 2019 and January 2020, allowing the collection of 87 water samples. Among 57 samples of the first campaign, 15 (26.3%) were positive for *Francisella* sp., nine (15.8%) for *F. tularensis* and/or *F. novicida*, and four (7.0%) for *F. tularensis* subsp. *holarctica*. The percentages were 83.3%, 80.0%, and 13.3% for the 30 samples of the second campaign (Figure 3).

Conclusions

Our *in vitro* experiments suggest that amoebae promote *Francisella* sp. survival in aquatic environments, including the tularemia agent *F. tularensis*. Our environmental study reveals a high prevalence of *Francisella* sp., including *F. tularensis,* in the studied aquatic environment. Altogether, our results suggest that the aquatic environment can be a natural reservoir of *F. tularensis*

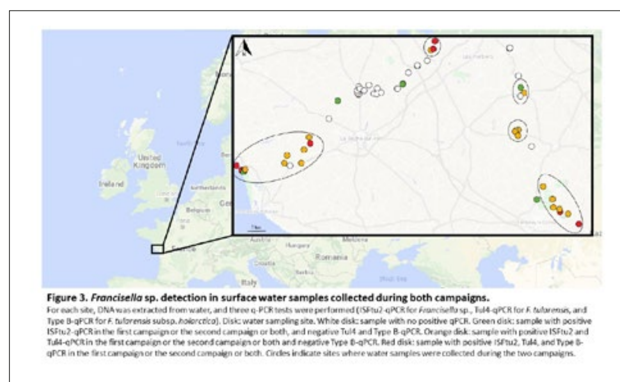

Figure 3. *Francisella* sp. detection in surface water samples collected during both campaigns.
For each site, DNA was extracted from water, and three q-PCR tests were performed (ISFtu2-qPCR for *Francisella* sp., Tul4-qPCR for *F. tularensis*, and Type B-qPCR for *F. tularensis* subsp. *holarctica*). Disk: water sampling site. White disk: sample with no positive qPCR. Green disk: sample with positive ISFtu2-qPCR in the first campaign or the second campaign or both, and negative Tul4 and Type B-qPCR. Orange disk: sample with positive ISFtu2 and Tul4-qPCR in the first campaign or the second campaign or both and negative Type B-qPCR. Red disk: sample with positive ISFtu2, Tul4, and Type B-qPCR in the first campaign or the second campaign or both. Circles indicate sites where water samples were collected during the two campaigns.

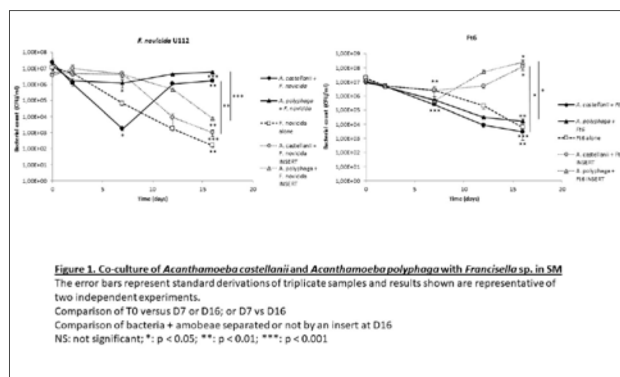

Figure 1. Co-culture of *Acanthamoeba castellanii* and *Acanthamoeba polyphaga* with *Francisella* sp. in SM
The error bars represent standard derivations of triplicate samples and results shown are representative of two independent experiments.
Comparison of T0 versus D7 or D16; or D7 vs D16
Comparison of bacteria + amobeae separated or not by an insert at D16
NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

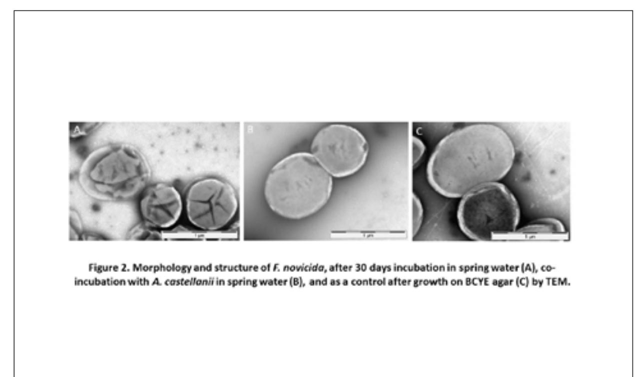

Figure 2. Morphology and structure of *F. novicida*, after 30 days incubation in spring water (A), co-incubation with *A. castellanii* in spring water (B), and as a control after growth on BCYE agar (C) by TEM.

P-56 Molecular-phylogenetic analysis of Anaplasma species in wildlife reveals the presence of zoonotic strains in North-Western Spain

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Background

Anaplasma species are widely distributed tick-borne bacteria of human and veterinary interest. Wild animals can be infected with *Anaplasma* spp., playing an important role in the epidemiology of anaplasmosis, not only as potential reservoirs of the pathogen but also in maintaining and spreading tick populations. Asturias (NW Spain) shows high abundance of *Ixodes ricinus* ticks and large populations of wild vertebrates in close contact with livestock farming, which increases the risk of infections for both domestic animals and humans. Our aim was to study the presence of potential zoonotic *Anaplasma* strains in wildlife species in this region.

Method

The presence of *Anaplasma* spp. was screened in spleen samples from ungulates (n= 31) and carnivores (n=76) collected between 2011-2019 by specific RT-PCR. Positive samples (11.2%) were further characterize using 16S rRNA and *groEL* genetic markers.

Results

Based on the 16S rRNA gene sequencing three *Anaplasma* species were identified: *A. phagocytophilum* was identified in cervids and one wolf, *A. capra* in one red deer and one fallow deer and *Anaplasma bovis* in one fallow deer. In addition, two *A. phagocytophilum* variants were found in cervids: variant “B”, usually associated with human cases and variant “W”, which is known to cause disease in livestock; while variant “I” was identified in wolf for the first time. Sequencing of the *groEL* gene, revealed the presence of the ecotype I, which is associated with the broadest host range and includes strains causing disease in domestic animals and humans.

Conclusions

The detection of the new zoonotic species *A. capra* in ungulates collected in 2013 evidences the presence of this pathogen in Europe prior to its first description in Asia. Furthermore, this is the first description of *A. capra* in fallow deer. These results contribute to the understanding of the role of wildlife in the eco-epidemiology of anaplasmosis in North-Western Spain.

Presence of *Anaplasma* species and the occurrence of *A. phagocytophilum* variants/ecotypes in spleen samples from wildlife in this study.

Host	Species	No. positive samples/ total tested (%)	*Anaplasma* species (n)	*A. phagocytophilum* 16S rRNA gene[a] (n)	*A. phagocytophilum* *groEL* gene[b] (n)
Ungulates	*Capreolus capreolus*	1/1 (100)	*A. phagocytophilum* (1)	variant I (1)	ND[c]
	Cervus elaphus hispanicus	7/7 (100)	*A. phagocytophilum* (6), *A. capra* (1)	variant W (4), variant B (2)	ecotype I (3), ND (3)
	Dama dama	3/3 (100)	*A. phagocytophilum* (1), *A. capra* (1), *A. bovis* (1)	variant W (1)	ND
	Sus scrofa	0/20 (0)			
Carnivores	*Canis lupus*	1/20 (5)	*A. phagocytophilum* (1)	variant I (1)	ecotype I (1)
	Vulpes vulpes	0/1 (0)			
	Felis silvestris	0/2 (0)			
	Meles meles	0/43 (0)			
	Mustela erminea	0/1 (0)			
	Martes martes	0/2 (0)			
	Mustela putorius	0/1 (0)			
	Neovison vison	0/2 (0)			
	Lutra lutra	0/3 (0)			
	Genetta genetta	0/2 (0)			
Total	**14**	**12/107 (11.2)**	***A. phagocytophilum* (9), *A. capra* (2), *A. ovis* (1)**	**variant W (5), variant B (2), variant I (1)**	**ecotype I (4)**

[a] 16S rRNA gene variants of *A. phagocytophilum* compared with human granulocytic anaplasmosis agent (GenBank U02521). Nomenclature used in previous studies (Silaghi et al., 2011; Overzier et al., 2013).

[b] *groEL* ecotypes of *A. phagocytophilum* compared to HGA agent (GenBank AF033101) according to the classification proposed by Jahfari et al. (2014).

[c] ND: not determined

P-57 First serological evidence of Q fever circulation in ruminant herds in French Guiana

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Background
Mammals are potential hosts and shedders of *Coxiella burnetii*, a ubiquitous and environnement-persistent bacterium responsible for Q fever zoonosis. Whereas the highest human Q fever incidence rate is recorded, some questions remain concerning the animal reservoir in French Guiana, especially since past studies had excluded livestock as a potential source of human infection.

Method
A Q fever seroprevalence study was conducted among 1228 sera from 86 cattle, goat, and sheep farms in main ruminant breeding area. Sera were collected from 2015 to 2017, then analyzed by Indirect ELISA (Priocheck Kit, ThermoFisher). Apparent prevalence was calculated based on the supplier's positivity threshold of 40% optical density ratio (ODR). A latent class model, obtained from French data, defined a relevant 8% ODR positivity threshold adapted to Guianese results distribution and estimated the kit sensitivity and specificity. Then, true prevalences were calculated both at individual and inter-herd levels.

Results
Manufacturer's 40%ODR threshold revealed a 0.6 to 2.3% apparent prevalence. However, the real prevalence calculated at the 8%ODR threshold was 14.2%, 1.8% and 3.7% respectively, for cattle, goats and sheep. The highest real prevalences appear in 2016 with 28% in cattle and 16.2% in goats. Inter-farm exposure rate was 66.2%, exposed farms were distributed in all agricultural areas studied.

Conclusions
Appropriateness of positivity threshold is a key in seroprevalence studies data analysis. Sensitivity and specificity applied in this study significantly revealed the positive serological status of animals. This study, based on a large sample, reveals for the first time the circulation of Q fever in livestock showing spatiotemporal variation. The herd exposure rate indicates that the role of livestock as an animal reservoir of *C. burnetii* should be taken into account for the control measures of this zoonosis in French Guiana, contrary to what has been accepted until now.

P-58 **Diversity of intracellular bacteria in fleas (Siphonaptera) infesting small mammals in Slovakia (Central Europe)**

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Background

Fleas (Siphonaptera) are obligate, blood-feeding ectoparasites and vectors of pathogens of medical and veterinary importance. Fleas are known vectors of *Rickettsia typhi* causing murine typhus and *Bartonella henselae* causing cat scratch disease. They also play a role in the transmission of epidemic typhus caused by *R. prowazekii.* Flea-borne spotted fever and its agent *R. felis* occur throughout the world. This study was aimed to examine fleas collected from wild rodents in Slovakia for the presence and diversity of *Rickettsia* and *Bartonella* species.

Method

A total of 640 rodents of six species were live-trapped in suburban, natural, and rural habitats and examined for the presence of ectoparasites. In total, 665 flea specimens were analysed. DNA from fleas was extracted using commercial kits. Sets of primers for PCR amplification of the *gltA*, 17-kDa antigen gene, *ompA*, and gene D were used to identify the species of *Rickettsia*. The presence of *Bartonella* spp. DNA was examined by a PCR assay targeting the 16S–23S rRNA gene intergenic spacer region. Positive amplicons were sequenced.

Results

The most common pathogen in fleas was *Bartonella* spp. (33.98%), followed by *Rickettsia* spp. (19.25%). *Bartonella* strains belonging to *B. taylorii, B. grahamii, B. elizabethae, Bartonella* sp. wbs11 and *B. rochalimae* clades were identified in *Ctenophthalmus agyrtes, C. congener, C. assimilis, C. sciurorum, C. solutus, C. bisoctodentatus, Palaeopsylla similis, Megabothris turbidus* and *Nosopsyllus fasciatus* from all habitats. The presence of *Rickettsia helvetica*, *R. monacensis,* rickettsiae belonging to *R. akari* and *R. felis* clusters, and endosymbionts with 96-100% identity to *Rickettsia* endosymbiont of *Nosopsyllus laeviceps laeviceps* were revealed in *C. agyrtes, N. fasciatus*, *C. solutus*, *M. turbidus, C. assimilis,* and *C. congener.*

Conclusions

The results suggest that the identified bacteria circulate in natural foci of Slovakia and fleas are their vectors or carriers. The study was financially supported by projects Vega 2/0021/21, APVV-19-0066, APVV-19-0519.

P-59 Evaluation using latent class models of the diagnostic performances of three ELISA tests commercialized for the serological diagnosis of Coxiella burnetii infection in domestic ruminants

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Background

Q fever is a worldwide zoonosis mainly responsible for reproductive disorders such as abortion in domestic ruminants and capable of infecting and circulating among a wide variety of animal species. Humans can be infected through exposure to contaminated aerosols from ruminants. ELISA methods are the diagnostic tools recommended for the serological diagnosis of *Coxiella burnetii* infection in ruminants but their respective diagnostic performances are difficult to assess because of the absence of a gold standard. This study focused on three commercial ELISA tests with the objectives to (1) assess their sensitivity and specificity in sheep, goats and cattle, (2) assess the between- and within-herd seroprevalence distribution in these species, accounting for diagnostic errors, and (3) estimate optimal sample sizes considering sensitivity and specificity at herd level.

Method

We comparatively tested 1,413 cattle, 1,474 goat and 1,432 sheep serum samples collected in France. We analyzed the cross-classified test results with a hierarchical zero-inflated beta-binomial latent class model considering each herd as a population and conditional dependence as a fixed effect. Potential biases and coverage probabilities of the model were assessed by simulation.

Results

Conditional dependence for truly seropositive animals was high in all species for two of the three ELISA methods. Specificity estimates were high, ranging from 94.8% [92.1;97.8] to 99.2% [98.5;99.7], whereas sensitivity estimates were generally low, ranging from 39.3 [30.7;47.0] to 90.5% [83.3;93.8]. Between- and within-herd seroprevalence estimates varied greatly among geographic areas and herds. Overall, goats showed higher withinherd seroprevalence levels than sheep and cattle. The optimal sample size maximizing both herd sensitivity and herd specificity varied from 3 to at least 20 animals depending on the test and ruminant species.

Conclusions

This study provides better interpretation of three widely used commercial ELISA tests and will make it possible to optimize their implementation in future studies.

P-60 Coxiella burnetii within and between-herd true seroprevalence assessment in domestic ruminants in France accounting for diagnostic uncertainty with latent class models

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Background
In 2017, the results of a large epidemiological investigation performed on 9,972 cattle, 5,024 goats and 7,632 sheep, aiming at assessing the seroprevalence of *Coxiella burnetii* in France, were published assuming that the ELISA test used was perfect. However, a recent study about the diagnostic accuracy of the three ELISA tests currently commercialized showed that the ELISA test used in this study is moderately sensitive (ranging between 54% and 75%) and that its specificity is inferior to 100% (ranging between 97% and 99%). Because the assumption of perfect sensitivity and specificity assumed in the original study could have led to under or overestimating within and between-herd seroprevalence, we reassessed here true seroprevalence levels in each species and estimated the importance of two potential risk factors (herd size and type of production) while accounting for the diagnostic uncertainty of the ELISA test used.

Method
We developed and implemented in a Bayesian framework a hierarchical logistic model that takes into account both within and between-herd variability on the seroprevalence. Diagnostic performances of the ELISA test used were considered in the binomial part of the model. We applied this model on the serological results of the original study.

Results
The proportion of truly seropositive herds was higher in dairy (median 47.9%) than in meat cattle herds (median 5.7%) and when the herd size was higher in cattle and goat herds. The proportion of truly seropositive animals in seropositive herds was higher in goats (median 63.6%) than in other species (median ranging from 22.9% and 39.4% respectively in cattle and sheep herds).

Conclusions
This study provides new insight related to the epidemiology of *Coxiella burnetii* in domestic ruminants in France. It allowed the identification and the quantification of risk factors related to the type of production and herd size.

P-61* Interaction of human neutrophils with Legionella pneumophila in a potential infection

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Background

Legionella pneumophila is a facultative intracellular bacterium that occurs worldwide and inhabits lakes, rivers, hot springs, and poorly maintained artificial water systems. *L. pneumophila* infects alveolar macrophages and causes Legionnaire's disease, an atypical pneumonia that can become fatal. Neutrophils infiltrate into the lungs of infected patients and attack bacteria. Despite their contribution, very little is known on the immune responses that occur during a *L. pneumophila* infection as well as the mechanisms that are being used. The objective of this study is to enlighten and understand the interaction of neutrophils with *L. pneumophila*.

Method

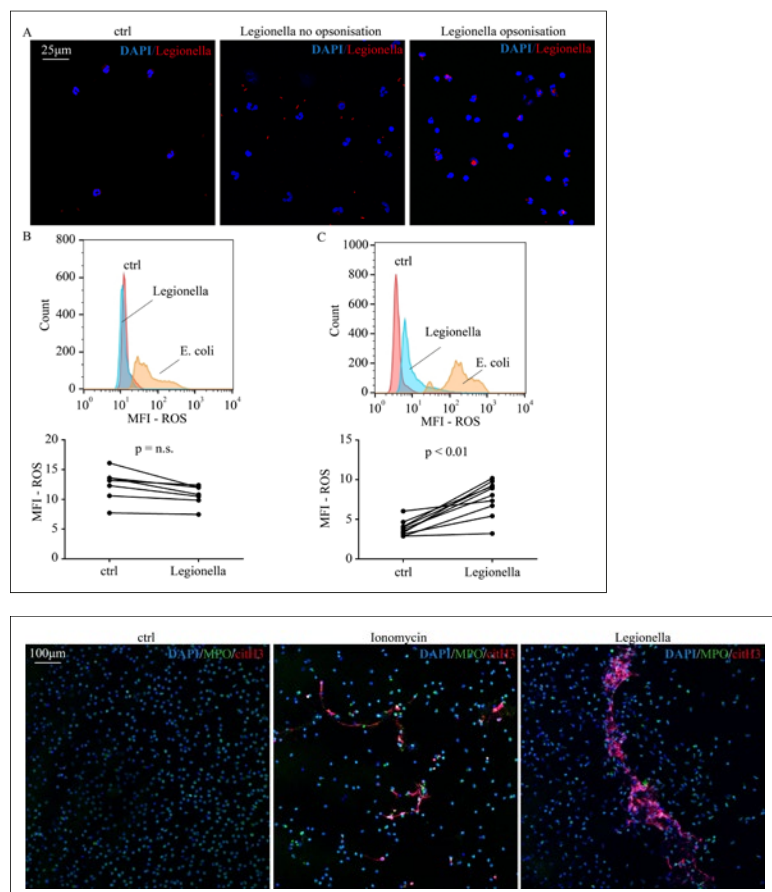
Neutrophils were isolated from heparinized blood of healthy individuals and infected with opsonized, agarderived *L. pneumophila* in a concentration of ~10 bacteria per neutrophil. After 3.5 hours of incubation, cells were fixed with 4% PFA. Immunolabeling was conducted to determine the ability of neutrophils to create Neutrophil Extracellular Traps (NETs). Furthermore, *Legionella* bacteria were immunolabeled to investigate if phagocytosis occurs. Finally, Reactive Oxygen Species (ROS) were also measured using flow cytometry.

Results

Neutrophils are able to phagocytose *Legionella* bacteria after opsonization, since nonopsonized bacteria are phagocytosed only to a very small extend in comparison. Moreover, ROS generation is induced by *Legionella* bacteria in neutrophils in a phagocytosis-dependent manner, since non-opsonized bacteria lose the capability to induce ROS. Finally, *Legionella* bacteria are able to induce NET release in neutrophils.

Conclusions

We provide evidence that in case of a *Legionella* infection, neutrophils fight invaders with phagocytosis, ROS generation and NET release. These findings can provide a springboard to investigate the impact of neutrophils to neutralize *Legionella* infection. In addition, determination of specific proteins present on NETs could be crucial in order to understand mechanisms/targets for the effective *Legionella* restrain.



*Student paper

P-62 Development of an Iron electrochemical sensor to monitor and control the presence of Legionella pneumophila in cooling towers

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Background

Legionnaires' disease is a human respiratory infection with a direct impact on infected people and indirectly responsible for a huge economic burden estimated at 1 billion per year in Europe. The disease is caused by *Legionella spp.* bacteria. These bacteria are basically found in freshwater environments including lakes and streams and they find a favourable growing environment in all human-made building water systems. *Legionella*is a chameleon able to persist in the environment in a free-living planktonic form but can also make biofilm to adhere and colonise surfaces. Bacterial pathogenesis is directly related to its facultative intracellular lifestyle and its ability to infect human cells. Iron has been described as an essential nutrient for *Legionella spp.* growth and replication. However, the role of iron in *Legionella* biofilm development is controversial, as well as the role of iron in bacterial persistence and growth within its host cells.

Method

We have developed an iron electrochemical sensor we can use to monitor the presence and quantity of iron in both ferrous Fe(II) and ferric Fe(III) states. This new tool is used to assess the role of both iron from in *Legionella pneumophila* behavior in biofilm.

Results

In environmental conditions, the ferrous Fe(II) iron is oxidized in ferric Fe(III) iron. Ferric Fe(III) iron is responsible of a decrease in *Legionella pneumophila* population (viability and growability of bacteria) within the biofilm but it does not inhibit biofilm formation. Also, the difference observed in between the decrease of cultivable bacteria (CFU) and the lower decrease of the total number of bacteria (genome copy number) suggests the presence of viable but non-culturable (VBNC) bacterial form.

Conclusions

These data shed light on the potential key role of iron in *Legionella pneumophila* behaviour associated to pathogenesis and give new opportunities towards understanding and control *Legionella spp.* growth and spread.

P-63* The localization and function of ubiquitin-modifying enzymes of Simkania negevensis

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Background

Simkania negevensis (*Sne*) is an obligate intracellular, *Chlamydia*-like bacterium that exhibits a biphasic developmental cycle with alternating differentiation between extracellular, non-replicative elementary bodies (EBs) and intracellular, replicative reticulate bodies (RBs). After the EBs invade into the host cell, they differentiate into RBs and multiply within a *Simkania* Containing vacuole (SnCV). In the early stages of infection, the autophagy marker and ubiquitin-like modifier LC3 is present on the SnCV surface. In order to survive within the host cell, intracellular pathogens have to avoid host cell responses such as the ubiquitination of the bacteriacontaining vacuole. So far, little is known about the interaction between *Sne* and the host ubiquitin system.

Method

Expression experiments, immunofluorescence microscopy and western blot were performed to analyze the localization of a *Sne* Ring-ligase in host cells. The ligase was purified and its activity was tested by autoubiquitination assays. To determine the influence of LC3 on *Sne* infection, infected cells were incubated with LC3 activity-based probes and pull down and mass spectrometry were performed.

Results

In a bioinformatic screen of the *Sne* genome a surprisingly high number of putative ubiquitin-modifying enzymes could be identified. In a previously published proteomics analysis of infected HeLa229 cells, three of the ubiquitin-related components could be detected. One of these components was later classified as a Ring-ligase. Since the enzyme was found in the proteome of the SnCV/ER membrane, its transport to the surface of the SnCV is likely occuring. After we analyzed the localization of the ligase in the cytoplasm of the host cell, the protein was successfully purified and first activity assays were performed. In ongoing experiments, cellular targets of the ligase will be identified. In initial tests, interaction partners between LC3 activity-based probes and *Sne* proteins were analyzed.

Conclusions

Our observations suggest an interaction between the host ubiquitin system and *Sne*.

*Student paper

P-64* Temperature sensitivity limits the host range of Rhabdochlamydia porcellionis

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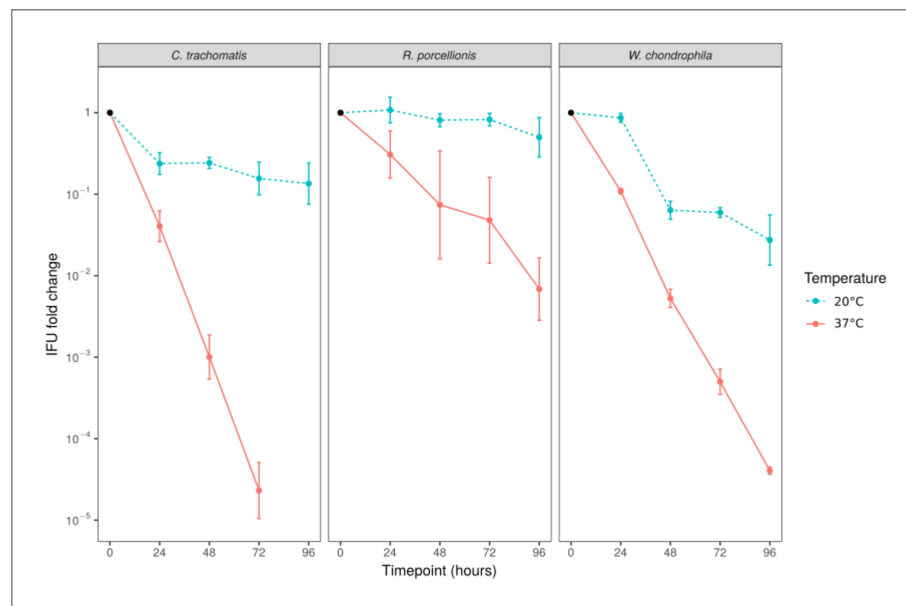
Background
The *Rhabdochlamydiaceae* family is a recent addition to the *Chlamydiales* order. Its members were initially discovered in cockroaches and woodlice, but were later detected in soil and freshwater environments and in patients suffering of respiratory infections. Unlike most chlamydia-like organisms, the *Rhabdochlamydiaceae* could not be propagated in amoebae and their host range remains largely unknown. We aimed to study the host range of *Rhabdochlamydia porcellionis,* the only cultured representative of the *Rhabdochlamydiaceae* family.

Method
We tested the permissivity of several mammal and arthropod cell lines: pneumocytes (A549), endothelial cells (HUVEC), endometrial cells (Ishikawa), fibroblasts (McCoy), *Aedes albopictus* larva cells (c6/36), *Ixodes ricinus* embryo cells (IRE/CVMT19) and *Spodoptera frugiperda* ovary cells (Sf9). The growth was measured by qPCR. To test the effect of temperature on elementary bodies (EB), the inclusion forming units were measured after incubation at 37°C and 20°C in cell-free medium. The growth was also assessed in Sf9 and A549 at 37°C and 28°C, respectively.

Results
A growth was observed only in Sf9 cells, with a doubling time of 23.3h. *R. porcellionis* did not grow in Sf9 incubated at 37°C, while *Waddlia chondrophila* grew in identical conditions. Switching from 28°C to 37°C at 2 days post-infection also stopped the replication. *R. porcellionis* EBs lost their infectiousness faster at 37°C than at room temperature, but the same effect was observed with *C. trachomatis* and *W. chondrophila* (Fig 1.).

Conclusions
The host range of *R. porcellionis* appears to be restricted by both temperature and tissue tropism. As *R. porcellionis* was only observed in the digestive tract and gonads of arthropods, the absence of growth in most arthropod cell lines is likely due to a limited tissue tropism. Moreover, the detrimental effect of an incubation at 37°C likely precludes any growth in mammals.



*Student paper

P-65* Tuberculosis: performance and modeling of the interdependence between microbiological test results and patient characteristics

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Background

Microbiological diagnosis of tuberculosis remains difficult and is based on three main tests aimed at identifying M. tuberculosis in clinical samples of patients: microscopy, PCR and culture. The objective of this study was to analyse the dependence between different microbiological tests and patient characteristics in order to propose models for predicting tuberculosis.

Method

We relied on a database of results obtained in our laboratory for 14,369 patients (472 positive for tuberculosis) or 34,565 tests (2009-2018). After an exploratory analysis of the data we have created a logistic regression model and a decision tree model to predict the result of the culture from the following data: year of analysis, age, sex, microscopy, PCR, type, appearance and number of leukocytes from the sample.

Results

The exploratory analysis allowed to determine the evolution of the number of tests per year and their overall performance using the culture as a reference. The sensitivity and specificity of microscopy were 51.3% and 99.3% for respiratory samples and 19.7% and 99% for nonrespiratory samples. The sensitivity and specificity of PCR was 80.5% and 100% for respiratory samples and 80% and 99.9% for nonrespiratory samples. The logistic regression model provided sensitivity and specificity of 81% and 100% on respiratory samples and 77.8% and 94.9% on nonrespiratory samples. The decision tree model provided sensitivity and specificity of 89.4% and 93.5% on respiratory samples and 95.8% and 90.7% on nonrespiratory samples.

Conclusions

This study confirms the added value of PCR due to a sensitivity and specificity far superior to microscopy and a considerable time saving in comparison with culture (which nevertheless remains the reference). By driving a decision tree model with a limited number of patient characteristics it is possible to improve the sensitivity of PCR. This opens up promising prospects if more clinical and epidemiological information were integrated into this model.

*Student paper

P-66 **Bacterial communities in association to specific tissues in wild morels**

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Background
The fungal genus *Morchella* is an iconic fungal group with great ecological and economic importance. Because of their widespread consumption, they represent some of the most emblematic fungal groups known to humankind

Method
In this study the bacterial communities associated with different types of tissues (fruiting bodies, mycelium, and sclerotia) in a diverse collection of morels (including wild individuals) were investigated. The fungi collected included representatives of the black and yellow morel clades

Results
Regardless of the species investigated, *Pseudomonas* spp. were detected as the most prevalent associate bacterium in mycelium and sclerotia. Together with *Ralstonia* spp. (mycelium and sclerotia) and *Methylobacterium* spp. (sclerotia), they represented the core associated bacterial community. In contrast, a highly diverse bacterial community was found associated with fruiting bodies, with representatives of *Pedobacter* spp., *Deviosa* spp. and *Bradyrhizobium* spp. constituting the core bacterial community. Multiple strains from Pseudomonas spp. were isolated from mycelia during the cultivation process. Confrontation assays with *Morchella* spp. for these *Pseudomonas* spp. resulted in multiple types of positive or negative interactions. The sequencing of the genomes of these *Pseudomonas* spp. allowed the identification of gene clusters relevant to promote positive interactions with morels, including secretion systems and toxin-antitoxin systems

Conclusions
This study offers the first *in-vivo* evidence linking observations from soils and confrontation studies suggesting the relevance of *Pseudomonas* spp. on the physiology and development of morels

P-67 Importazole, a specific inhibitor of importin-dependent nuclear transport, suppresses growth of Anaplasma phagocytophilum in HL-60 cells by preventing AnkA nuclear accumulation

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Background

Anaplasma phagocytophilum (AP) is a gram-negative obligate, tick-borne bacterium of neutrophils that causes human granulocytic anaplasmosis. Ankyrin A (AnkA), an ankyrin repeat protein essential for bacterial growth is delivered into the host cytoplasm by a type IV secretion system. After injection, AnkA translocates into the nucleus, binds to host DNA and reprograms epigenetics of the host cell to promote bacterial survival and growth.

Method

In this study, we utilized a small molecule importazole, a specific inhibitor of the importin-dependent nuclear transport system, to investigate its effects on AP growth inside the infected HL-60 cells.

Results

By using qPCR, we found that infected cells had 175 + 75 bacteria per cell (mean + SEM) after 48-72 hours post infection and showed significant decrease in host cell viability from 91+ 5% to 63 + 3%, $p < 0.01$, measured by trypan blue exclusion method and automatic viability cell counter. In contrast, we found that incubation with 10 μM importazole strongly suppressed AP growth, to 15 + 7 bacteria per cell, and significantly improved viability of the infected cells, from 63% to 79+2% ($p < 0.05$). Furthermore, by using fluorescent microscopy-based colocalization analysis and cell transfection with eGFP-labeled AnkA plasmid constructs we also found that importazole significantly reduced nuclear localization of the AnkA in the model transfected HEK293 cells. In addition, when the cells were loaded with GTPγS, a non-hydrolysable analog of GTP that inhibits GTP-dependent cellular processes, including RanGTP-dependent nuclear protein transport, we observed a similar inhibitory effect on AP growth in the infected HL-60 cells and on the nuclear localization of eGFP-AnkA in the transfected HEK293 cells.

Conclusions

These data show that AP infection and growth depend on AnkA translocation into the host nucleus through importin/RanGTP-dependent nuclear transport system.

P-68 **Cellular effects of fosfomycin treatment on *Wolbachia* spp.**

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Background

Filarial nematodes infect about 100 million humans and can cause lymphatic filariasis (Elephantiasis) or onchocerciasis (River Blindness). Since *Wolbachia* spp., obligate endobacteria of filarial worms, are essential for embryogenesis, larval development and adult survival, anti-wolbachial therapy is a potent treatment of filarial infections. Fosfomycin inhibits the first dedicated cytoplasmic enzyme for lipid II synthesis (MurA) and is effective against many antibiotic-resistant bacteria. Although *Wolbachia* have a reduced genome and lack a canonical cell wall, key enzymes needed to synthesize lipid II, including MurA, are conserved. Fosfomycin treatment leads to enlarged *Wolbachia* cells, demonstrating that lipid II is necessary for cell division.

Methods

To elucidate the cellular effects of fosfomycin treatment, *Wolbachia*-infected C6/36 insect cells were treated for 9 days. Total RNA and proteins were extracted and will be used for transcriptomics and proteomics to characterize the expression levels of fosfomycin-treated *Wolbachia*. Pathways of interest, besides cell wall biosynthesis and cell division, are branched fatty acid synthesis, starvation and oxidative stress phenotypes shown to be upregulated in other fosfomycin-treated bacteria, and stress-response elements.

Results

Different assay setups and RNA preservation methods led to the identification of conditions yielding high-quality RNA for RNA-Seq. Depletion of rRNA with riboPOOLs was found to work best and was combined with insect mRNA depletion using Dynabeads to increase coverage of the wolbachial transcripts; RNA-Seq is running. For proteomics, 80% coverage was achieved with an optimized extraction method from *Wolbachia*-enriched samples. Wolbachial stress-response genes will be measured using RT-qPCR, and the *Wolbachia* phenotype after antibiotic removal will be characterized using fluorescence microscopy.

Conclusions

These analyses will clarify if depletion of *Wolbachia* by fosfomycin is the result of active damage or the failure of cells to divide. Understanding the *Wolbachia* spp. cellular response to fosfomycin will provide insight into its mechanism of action in this reduced-genome endosymbiont.

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