ESCCAR - ASR JOINT MEETING
International Congress on Rickettsiae and other Intracellular Bacteria

Book of Abstracts

June 19th to 21st 2017
IHU Méditerranée Infection
Marseille, France
Dear colleagues, Dear friends,

I am extremely pleased and honored to welcome you in Marseille for the ESCCAR-ASR joint meeting 2017 which marks the 50th anniversary of international rickettsiology meetings. The adventure began with a first meeting in Slovakia in 1967 and the rhythm of meetings progressively increased to its current pace of one congress every three years. The 2017 meeting gathers more than 200 participants from throughout the world, and the program is equally balanced between oral and poster presentations. I would like to acknowledge the precious help of the members of the ESCCAR and ASR boards who helped me with the organization of these three dense days. I hope that you will enjoy your time in Marseille and wish you a fruitful conference.

Pierre-Edouard Fournier
ESCCAR president
Content

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**International Congress on Rickettsiae and other Intracellular Bacteria**

**Monday, June 19th, 2017**

- **08.35-08.50** Welcome address (Amphitheater Toga)
- **08.50-09.35** Keynote lecture (Amphitheater Toga)
- **09.35-09.55** Contribution of rickettsiology to modern microbiology: "The *Rickettsia felis* paradigm"  
  Didier Raoult (Marseille, France)
- **09.35-10.25** Session 1 - Part 1: *Rickettsia* epidemiology (Amphitheater Toga)  
  **Moderators:** O. Mediannikov & Loyd Vaughan
- **09.35-10.00** Use of MALDI-TOF MS to identify arthropod vectors of rickettsioses and to detect the microorganisms they carry  
  Maureen Laroche (Marseille, France)
- **10.00-10.25** The evolving mosaic of tick-borne rickettsioses in North America  
  Christopher D. Paddock (Atlanta, GA, USA)

**10.25-11.00** Coffee break and poster session 1

- **11.00-12.15** Session 2: Updates in the *Chlamydiaceae* field (Amphitheater Toga)  
  **Moderators:** Johannes Hegemann & Christopher D. Paddock
- **11.00-11.20** New insights in the biology of *Chlamydia*-related bacteria  
  Gilbert Greub (Lausanne, Switzerland)
- **11.20-11.45** Epidemiology, diversity and pathogenic role of *Chlamydia*-related bacteria  
  Mirja Puolakkainen (Helsinki, Finland)
- **11.45-11.55** Ticks as a new potential reservoir and vector for chlamydiae  
  Ludovic Pilloux (Lausanne, Switzerland)
- **11.55-12.05** Novel *Candidatus* Rhabdochlamydia sp. associated with both native Australian marsupials and their parasiting tick species  
  Delaney Burnard (Brisbane, Australia)
- **12.05-12.15** Antibiotic susceptibility of *Simkania negevensis*  
  Manon Vouga (Lausanne, Switzerland)
11.00-12.35 Parallel Session 2bis: Pathophysiology of Anaplasmataceae (Amphitheater IHU)

Moderators: Nahed Ismail & Yasuko Rikihisa

11.00-11.20 Ehrlichia exploitation of conserved host cell pathways for survival
Jere W. McBride (Galveston, TX, USA)

11.20-11.45 MyD88-Dependent mTORC1 activation Inhibits Autophagy and Elicits Excessive Inflammasome Activation during Fatal Ehrlichia Infection
Nahed Ismail (Pittsburgh, PA, USA)

11.45-11.55 Ehrlichia minasensis, a model organism to study bacterial host shift
Alejandro Cabezas-Cruz (Maisons-Alfort, France)

11.55-12.05 Ehrlichia chaffeensis tandem repeat effector targets differentially influence infection
Tian Luo (Galveston, TX, USA)

12.05-12.15 Human anti-OMP-1 monoclonal antibodies inhibit Ehrlichia chaffeensis infection through extracellular and intracellular mechanisms
Thangam Sucha Velayutham (Galveston, TX, USA)

12.15-12.25 Structural basis for recombinatorial permissiveness in the generation of Anaplasma marginale Msp2 antigenic variants
Telmo Graça (Pullman, WA, USA)

12.25-12.35 Exploration by a dual approach of the role in cattle abortion of an Anaplasma phagocytophilum vacuolar membrane protein
Anne-Claire Lagrée (Maisons-Alfort, France)

12.35-14.00 Lunch and poster session 1

14.00-15.10 Session 3: Bartonella epidemiology (Amphitheater Toga)

Moderators: Jane Koehler & Beate Heinrichreise

14.00-14.25 Bartonella henselae and cat scratch disease: from epidemiology to genomics in a “one health” perspective
Henri-Jean Boulouis (Maisons-Alfort, France)

14.25-14.50 Bartonella: from cats to bats
Bruno Chomel (Davis, CA, USA)

14.50-15.00 Prevalence (culture, PCR and serology) of Bartonella spp. in asymptomatic people working in a sanitary center from La Rioja, Spain
Aranzazu Portillo (Logrono, Spain)

15.00-15.10 Understanding the influence of host demography on Bartonella diversity
Richard Hassall (Aberdeen, United Kingdom)

14.00-15.25 Parallel session 3bis: Genomics and genetics of Chlamydiales (Amphitheater IHU)

Moderators: Adam Polkinghorne & Matthieu Million

14.00-14.25 Genomic analyses provide insight into the evolution and adaptation of intracellular bacteria within phylum Chlamydiae
Adam Polkinghorne (Brisbane, Australia)

14.25-14.50 The chlamydial plasmid: role in tropism and virulence
Ian Clarke (Southampton, United Kingdom)

14.50-15.15 Transformation of chlamydia: current approaches and impact in our understanding of chlamydial biology
Kenneth Fields (Lexington, KY, USA)

15.15-15.25 Comparative genomics of chlamydiae
Trestan Pillonel (Lausanne, Switzerland)
15.10-16.15 Coffee break and poster session 1

16.15-17.50 Session 4: Pathogenesis of Chlamydia and Chlamydia-related bacteria

Moderators: Steven Webster & Jason Carlyon

16.15-16.40 Chlamydia-related bacteria and related epitheliocystis in fish
Loyd Vaughan (Zurich, Switzerland)

16.40-17.05 Targeting epidermal growth factor signaling is critical to inclusion development in Chlamydia trachomatis infection
Allen W. Tsang (Winston-Salem, NC, USA)

17.05-17.30 Genetic dissection of Chlamydia virulence factors
Raphael Valdivia (Raleigh, NC, USA)

17.30-17.40 Experimental challenge of pregnant cattle with the putative abortifacient Waddlia chondrophila
Nick Wheelhouse (Edinburgh, United Kingdom)

17.40-17.50 An homologue of a sporulation protein of B. subtilis is involved in peptidoglycan remodeling during chlamydial division
Nicolas Jacquier (Lausanne, Switzerland)

17.50-18.30 Session 1 - Part 2: Rickettsia epidemiology (Amphitheater Toga)

Moderators: O. Mediannikov & Loyd Vaughan

17.50-18.00 First detection of Rickettsia monacensis in Denmark
Silke Wöfel (Munich, Germany)

18.00-18.10 Rickettsia parkeri in Amblyomma maculatum (Acari : Ixodidae) collected from multiple locations in southern Arizona
Michelle E.J. Allerice (Atlanta, GA, USA)

18.10-18.20 Rickettsia species in ectoparasites collected from small rodents in Lithuania
Dalyte Mardosaite-Busaitiene (Kaunas, Lithuania)

18.20-18.30 Diversity of Rickettsia in ticks collected in Central Italy 2014-2016
Ilaria Pascucci (Teramo, Italy)

19.00-20.00 Jazz concert (Amphitheater Toga)
The CNRS big band

20.00-22.00 Welcome drink (Amphitheater Toga)
Tuesday, June 20th, 2017

08.30-10.05 Session 5: Other fastidious pathogens (Amphitheater Toga)
  Moderators: Gilbert Greub & Jose Antonio Oteo
08.30-08.55 Tularemia: changing epidemiological patterns in the world
  Max Maurin (Grenoble, France)
08.55-09.20 From bacteria to ticks: lessons in antimicrobial defense
  Seonmy Chou (San Francisco, CA, USA)
09.20-09.45 Emerging horizons for tick-borne pathogen research: from the one pathogen-one
  disease vision to the pathobiome paradigm
  Alejandro Cabezas-Cruz (Maisons-Alfort, France)
09.45-09.55 Comparative analysis of Mycobacterium kansasii subtypes
  Florian Tagini (Lausanne, Switzerland)
09.55-10.05 New European tick cell lines for isolation and propagation of intracellular bacteria
  Lesley Bell-Sakji (Liverpool, UK)

10.05-10.45 Coffee break and poster session 2

10.45-12.05 Session 6: Pathophysiology of Q fever (Amphitheater Toga)
  Moderators: Stacey D. Gilk & Alejandro Cabezas-Cruz
10.45-11.10 Cholesterol and loss of the Coxiella intracellular niche
  Stacey D. Gilk (Indianapolis, IN, USA)
10.45-11.35 Defining Coxiella-host cell interactions using a human lung infection platform
  Daniel E. Voth (Little Rock, AK, USA)
11.35-11.45 CXCL-9, a promising biomarker in the diagnosis of chronic Q fever
  Anne F.M. Jansen (Nijmegen, the Netherlands)
11.45-11.55 Development of anaerolos model of Q fever in the common marmoset
  (Callithrix jacchus)
  Dstj (Salisbury, UK)
11.55-12.05 Immunological manifestations in a chronic Q fever patient: a common problem with
  potential diagnostic and therapeutic pitfalls.
  Ruud P.H. Raijmakers (Nijmegen, the Netherlands)

10.45-11.20 Parallel Session 6bis: Type III secretion system of chlamydiae (Amphitheater IHU)
  Moderators: Daniel Paris & Stephen Graves
10.45-11.10 The type three secretion system of chlamydiae
  Agathe Subtil (Paris, France)
11.10-11.20 Identification and characterization of Wae1, W. chondriophila effector protein
  secreted by type III secretion system within host cell cytoplasm
  Carole Kebbi-Beghdadi (Lausanne, Switzerland)
11.20-12.10 Parallel Session 8or: Epidemiology of rickettsioses (Amphitheater IHU)
  Moderators: Daniel Paris & Stephen Graves
11.20-11.45 Transmission mechanisms for emerging rickettsial pathogens
  Kevin Macaluso (Baton Rouge, LA, USA)
11.45-12.10 Rickettsia sibirica mongolitimonae, a frequent rickettsiosis in France
  Emmanouil Angelakis (Marseille, France)
12.10-13.00 Lunch and poster session 2
13.00-15.05 Session 7: Genomics, transcriptomics and ecology of Anaplasmataceae  
(Amphitheater Toga)  
Moderators: J. Stephen Dumler & Mirja Puolakkainen

13.00-13.25 Anaplasm phagocytophilum AnkA nuclear architecture alterations and transcriptional reprogramming  
J. Stephen Dumler (Baltimore, MD, USA)

13.25-13.50 Roles and functions of T4SS in Ehrlichia chaffeensis  
Yasuko Rikihisa (Columbus, OH, USA)

13.50-14.15 A tick endosymbiont with Anaplasma marginale in cell culture  
Kelly A. Brayton (Pullman, WA, USA)

14.15-14.25 Genomic analysis of four Ehrlichia species identified large and diverse effector repertoires  
Christophe Noroy (Gaudeloupe, France)

14.25-14.35 Molecular approach for the discovery of unknown Anaplasmataceae species  
Mustapha Dahmani (Marseille, France)

14.35-14.45 Screening of tick-borne pathogens in Caribbean ticks using new high-throughput technologies (DOMOTICK project)  
Mathilde Gondard (Maisons-Alfort, France)

14.45-14.55 Prevalence of Anaplasma, Bartonella and Borrelia species in raccoon dogs (Nyctereutes procyonoides) in the Republic of Korea  
Young-Sun Cho (Seoul, Korea)

14.55-15.05 Detection of tick-borne diseases in wild boars (Sus scrofa) in the Republic of Korea  
Jeong-Byoung Chae (Seoul, Korea)

15.05-15.40 Coffee break and poster session 2

15.40-17.25 Session 8: Pathophysiology of Chlamydia (Amphitheater Toga)  
Moderators: Ian Clarke & Bruno Chomel

15.40-16.05 Adhesins of Chlamydia  
Johannes Heggemann (Dusseldorf, Germany)

16.05-16.30 Chlamydia and the inflammasome  
Steven Webster (Cambridge, United Kingdom)

16.30-16.55 New insights on the ultrastructure of Chlamydia and their interactions with the host cells  
Richard Hayward (London, United Kingdom)

16.55-17.05 Chlamydia energy metabolism revisited: VIA-ATPase in focus as a novel drug target  
Denys Pogoryelov (Frankfurt Am Main, Germany)

17.05-17.15 Identification of Waddlia chondrophila proteins involved in transcriptional regulation  
Marie de Bary (Lausanne, Switzerland)

17.15-17.25 Characterization of cell division mechanisms in the Chlamydia-related bacterium Waddlia chondrophila  
Firuza Bayramova (Lausanne, Switzerland)
15.40-17.25 Parallel session 8bis: Epidemiology and pathophysiology of ehrlichiae
(Amphitheater IHU)
Moderators: Jean-Marc Rolain & Allen W. Tsang
15.40-16.05 Epidemiology of infections caused by Anaplasmataceae in animals
Jean-Lou Marié (Toulon, France)
16.05-16.30 Ecology of Anaplasma phagocytophilum informs its evolution
Janet E. Foley (Davis, CA, USA)
16.30-16.55 Candidatus Neoehrlichia mikurensis in Europe
Jose Antonio Oteo (Logrono, Spain)
16.55-17.05 Candidatus Neoehrlichia mikurensis in patients with meningitis-like symptoms
after tick-bite in Poland
Anna Moniuszko-Malinowska (Bialystok, Poland)
17.05-17.15 Anaplasma phagocytophilum in Japan
Norio Ohashi (Shizuoka, Japan)
17.15-17.25 Prevalence of tick-borne pathogens in dogs from Korea
Jun-Gu Kang (Seoul, Korea)
19.00 Gala dinner (Fort Ganteaume)
08.30-10.10 Session 9: new aspects of Q fever (Amphitheater Toga)
Moderators: Emmanouil Angelakis & Richard Hayward
08.30-08.55 Outcome and complications of Q fever, lessons from the Dutch national database
Chantal Bleeke-Rovers (Nijmegen, The Netherlands)
08.55-09.20 New clinical forms of Q fever
Matthieu Million (Marseille, France)
09.20-09.30 Chronic Q fever-related complications and mortality: data from a nationwide cohort
Sonja E. van Roeden (Utrecht, the Netherlands)
09.30-09.40 Coxiella burnetii and Borrelia burgdorferi sensu lato in poultry red-mites,
Dermamyssus gallinae related to red-mite dermatitis outbreaks in city-dwellers, in Italy
Donato Antonio Raelle (Foggia, Italy)
09.40-09.50 Progression of Coxiella burnetii contamination in the air and environment
in naturally infected sheep flocks during two consecutive lambing seasons
Raquel Alvarez-Alonso (Bizkaia, Spain)
09.50-10.00 Abortion nor placental Coxiella burnetii excretion in pre-breeding challenged
pregnant goats
Hendrik-Jan Roest (Wageningen, the Netherlands)
10.00-10.10 Coxiella burnetii – epidemiological situation with particular emphasis on genotypes
circulating in population of Polish ruminants
Monika Szymanska-Czerwinska (Pulawy, Poland)

08.30-10.10 Parallel Session 9bis: Epidemiology and diagnosis of Rickettsioses
(Amphitheater IHU)
Moderators: Shuji Ando & Oleg Medniannikov
08.30-08.55 Update on vector-borne rickettsioses in North Africa
Idir Bitam (Alger, Algeria)
08.55-09.20 Diversity and uniformity of rickettsia and Rickettsia-related pathogens in Japan
Shuji Ando (Tokyo, Japan)
09.20-09.30 Eschar and multi-organ dysfunction: preliminary findings from India
John A.J. Prakash (Vellore, India)
09.30-09.40 Molecular and MALDI-TOF identification of ticks and tick-associated bacteria in Mali
Adama Zan Diarra (Bamako, Mali)
09.40-09.50 Evaluation of blood collection tube additives for Rickettsia rickettii conservation
and detection
Ida Chung (Atlanta, GA, USA)
09.50-10.00 Factors affecting the successful in vitro isolation of rickettsial organisms
from clinical samples
Matthew T. Robinson (Oxford, UK)
10.00-10.10 Exponential increases in Rickettsia spp. molecular detection sensitivity
Cecilia Y. Kato (Atlanta, GA, USA)

10.10-10.45 Coffee break and poster session 3

10.45-12.50 Session 10: Rickettsia, from genomics to diagnostics (Amphitheater Toga)
Moderators: Kevin Macaluso & Agathe Subtil
10.45-11.10 Rickettsial genomics and the paradigm of genome reduction associated
to increased virulence
Pierre-Edouard Fournier (Marseille, France)
11.10-11.35 New approaches in the systematics of rickettsiae
Stanislav Shpynov (Moscow, Russia)
11.35-12.00 Rickettsia philipii: its vector, disease, and genetics
Marina Eremeeva (Statesboro, GA, USA)
12.00-12.10 Retrospective analysis of tetracycline and new quinolone therapy for Japanese spotted fever
Kazuhiro Itch (Fukui, Japan)

12.10-12.20 Atg5-dependent autophagic response in macrophages supports the survival of Rickettsia australis via inhibiting IL-1β secretion
Rong Fang (Galveston, TX, USA)

12.20-12.30 Host players involved in the interaction between spotted fever group rickettsiae and macrophage-like cells
Simoes Isaura (San Francisco, CA, USA)

12.30-12.40 European isolate of Rickettsia felis from a tick
Monika Danchenko (Bratislava, Slovakia)

12.40-12.50 Three rickettsial genotypes detected in mosquitoes from the Republic of Korea
Allen L. Richards (Silver Springs, MD, USA)

12.50-14.00 Lunch and ESCCAR committee election and poster session 3

14.00-15.35 Session 11: Bartonella pathophysiology and ancient infections
( Amphitheater [IHU]
**Moderators: Allen L. Richards & Kenneth Fields**

14.00-14.25 Molecular adaptation of Bartonella to its human and louse niches
Jane E. Koehler (San Francisco, CA, USA)

Diana G. Scorpio (Baltimore, MD, USA)

14.50-15.15 Emerging Bartonella infections in West Africa
Oleg Medrannikov (Marseille, France)

15.15-15.25 Infections with Bartonella spp. in free ranging cervids and deer keds (Lipoptena cervi) in Southern Norway
Algimantas Paulauskas (Kaunas, Lithuania)

15.25-15.35 Detection and seroprevalence of Bartonella henselae in small Indian mongooses (Herpestes auropunctatus) from Grenada island
David A. Jaffe (Davis, CA, USA)

14.00-15.40 Parallel Session 11bis: Orientia tsutsugamushi (Amphitheater Toga)
**Moderators: Kelly Brayton & Raphael Vaidiva**

14.00-14.25 Molecular insights into how Orientia tsutsugamushi ankyrin repeat-containing effectors modulate host cell processes
Jason A. Carlyon (Richmond, VA, USA)

14.25-14.50 Clinical trial of doxycycline and azithromycin therapy in murine typhus therapy1
Paul Newton (Vientiane, Laos)

14.50-15.00 The burden of scrub typhus – the Asian perspective
Daniel Paris (Basel, Switzerland)

15.00-15.10 The ecology of scrub typhus – facts, fiction and filling in the gaps
Ivo Elliott (Oxford, UK)

15.10-15.20 Inhibition of RNA polymerase switch region by corallopyronin A: a new drug target against Orientia tsutsugamushi in vitro and in vivo
Christian Keller (Marburg, Germany)

15.20-15.30 Genetic diversity of Orientia tsutsugamushi strains from patients in north India
Manisha Biswal (Chandigarh, India)

15.30-15.40 Orientia tsutsugamushi modulates endoplasmic reticulum stress and endoplasmic reticulum-associated degradation for optimal growth
Kyle Rodino (Richmond, VA, USA)
15.40-16.15 Coffee break and poster session 3

16.15-17.20 Session 12: Coxiella burnetii vaccine, diagnosis and genomics (Amphitheater Toga)
Moderators: Idir Bitam & Dimitrios Frangoulidis

16.15-16.40 A new vaccine for Q fever
Stephen Graves (Geelong, Australia)

16.40-16.50 Elucidating and exploiting O-antigen biosynthesis for Q fever vaccine
Alice Cross (Exeter, UK)

16.50-17.00 Harmonization of PCR-based diagnosis for improved quality of data:
the example of the French network of Q fever surveillance in ruminants
Elodie Rouset (Sophia-Antipolis, France)

17.00-17.10 Swab cloths as tools to reveal Q fever environmental contamination in ruminant herds
Elsa Jourdain (Saint Genès Champanelle, France)

17.10-17.20 Comparison of the attenuated Nine Mile strain clone 4 (phase II) with strain Nine Mile phase I (RSA 493) at the genome level
Dimitrios Frangoulidis (Munich, Germany)

17.30-18.00 Young investigator awards and Farewell address (Amphitheater Toga)
ESCCAR-ASR
JOINT MEETING 2017

International Congress on Rickettsiae and other Intracellular Bacteria

POSTER SESSION 1 – JUNE 19th
10.20-10.55 / 12.40-14.00 / 15.25-16.15

P1-1) Anthony F. Barbet – Genome relationships between Anaplasma phagocytophilum strain

P1-2) Cho Yoon Kyoung – Experimental infection study in dogs with Anaplasma phagocytophilum Korean isolate

P1-3) Lee Seung Hun – Immune cell activation and proinflammatory responses in A. phagocytophilum of human isolate

P1-4) Nathalie Vachiery – Ehrlichia ruminantium detection using efficient high-throughput molecular method

P1-5) Monika Szymanska-Czerwinska – Free-living and captive turtles and tortoises as carriers of new Chlamydia spp.

P1-6) Ludovic Pilloux – Discovery of new virulence factors secreted by Chlamydiales bacteria

P1-7) Ludovic Pilloux – Chlamydia – related bacteria induce inflammasome activation

P1-8) Trestan Pillonel – Taxogenomics of uncultured chlamydiae

P1-9) Liudmila Rubanik – Determination of phenotypic and genotypic antibiotic resistance of Chlamydia trachomatis isolates

P1-10) Manon Vouga – A novel specific real time PCR reveals that Simkania negevensis is not an important pathogen in Western Europe.

P1-11) Heijne Marloes – OmpA sequencing of C. gallinacea-positive samples from layer hens

P1-12) Algimantas Paulauskas – New records of Bartonella in deer ked in Lithuania

P1-13) Meriem Louni – Bartonella quintana in Human body in Algeria : a risk of re-emergence of Trench fever?

P1-15) Juan Carlos Garcia – Hepatosplenic cat scratch disease in immunocompetent adults: our experience with 8 cases.

P1-16) Nadia Amanzougaghene – Detection of several emerging bacterial pathogens in human head lice from Mali.

P1-17) Ananda Müller – Molecular detection and characterization of Bartonella spp. and Rickettsia felis in fleas collected from domestic cats in Valdivia, Southern Chile.


P1-19) Laura Franzin – Preliminary results of intracellular bacteria detection from culture-negative endocarditis patients in North-West Italy.

P1-20) Sonia Santibanez - Rickettsia spp. detected in Dermacentor marginatus from DEBONE/L/TIBOLA/SENLAT patients.

P1-21) Emilie G. Clemenc- A prospective study of spotted fever group and typhus group rickettsioses as the etiology of undifferentiated fever in northern Sabah, Norveco, East Malaysia.

P1-22) Lukas Frans Ocias – Rickettsiosis in Danish patients suspected of Lyme neuroborreliosis.

P1-23) Rita de Sousa – A case of scrub typhus imported to Portugal, 2016.

P1-24) Aranzazu Portillo – A first look at the bacterial communities of four tick species that bite humans in La Rioja (North of Spain) using high-throughput sequencing.

P1-25) Fayaç Zeroual – Rickettsia slovaca in the spleen from wild boars, Algeria.

P1-26) Valentina Chisu – Molecular detection of Rickettsia species and first report of Rickettsia raoulti in ticks collected from domestic and wild hosts in Sardinia, Italy.

P1-27) Kati Hokynar – Chlamydia-like organisms (CLO) in skin biopsies from lesions of granuloma annulare and morphea.


P1-29) Le Jiang-Development of Sensitive and rapid recombinase polymerase amplification assay for the direct detection of Anaplasma phagocytophilum.

P1-30) Shubhajit Mitra - Polycomb group Protein recruitment and degradation promotes Ehrlichia infection and modulates hox gene expression and epigenetic marks.


P1-33) Michelangelo Autery - Rickettsia species in Italy.

P1-34) Michelangelo Autery - Detection of Bartonella spp. Ctenocephalides fleas from dogs in Southern Italy.

P1-35) Michelangelo Autery - Bartonella species from stray cats and dogs and their ectoparasites in Sicily.
P2-1) Amira Nebbak – Detection of bacteria in Ixodes ricinus ticks in the Alsace region, France

P2-2) Mammar Khames – Epidemiological situation of human brucellosis in Medea, Algeria

P2-3) Luc Chabanne – Bacterial microbiota associated with Rhipicephalus sanguineus s.l. ticks from France, Senegal and Arizona

P2-4) Sonia Santibanez – Isolation of Rickettsia amblyommatis in HUVEC cell line

P2-5) Malgorzata Kowalczewska – Protein markers for diagnosis of rickettsioses

P2-6) Alyssa Snellgrove – Effect of Rickettsia rickettsii infection on the biological parameters and survival of Amblyomma americanum

P2-7) Michael L. Levin – Reactivation of Rickettsia in the vector revisited: minimal duration of tick attachment necessary for transmission of infection.


P2-9) Frantisek Csicsay – Study of the antigenicity of Rickettsia akari proteins using proteomics approaches

P2-10) Rastislav Sorf – Rickettsial infection resulting to neuronal cell death is associated with activation of proinflammatory chemokines

P2-11) Ruud P.H. Rajmakers – Autoimmunity and cryoglobulinemia in chronic Q fever pose diagnostic and therapeutic challenges

P2-12) Sonja E. Van Roeden – Serum doxycycline concentration-based dosing in the treatment of chronic Q fever

P2-13) Gilbert J. Kersh – Antibiotic susceptibility of Coxiella burnetii in axenic media

P2-14) Anne F.M. Jansen – Failure of interferon-gamma treatment in a patient with chronic Q fever

P2-15) Stanislaw N. Shpynov – Nano-agglutination is express test for detection of antibodies to Coxiella burnetii and Rickettsia prowazekii

P2-16) Elsa Prudent – Fluorescence in situ hybridization: a complementary tool for Q fever diagnosis

P2-17) Ana M. Palomar – Investigation of Rickettsia spp., Anaplasma spp. And Coxiella burnetii in ticks removed from donkeys in South Africa

P2-18) Bernard Davoust – Survey on Coxiella burnetii infection in dogs, French Guiana

P2-19) Bernard Davoust – Identification of Coxiella burnetii in in fertile dairy cattle with chronic endometritis

P2-20) Selim Abdelfattah – Characterization of ruminant Coxiella burnetii field strains in a Galleria mellonella host-based Model
P2-21] Aurélien Joulié – In vivo and in vitro infectivity of Coxiella strains from French livestock

P2-22] Marcela Mori – Protective immunity induced by experimental phase I inactivated vaccines against sub-lethal challenge of Coxiella burnetii in vivo

P2-23] Eulalia Guerrini – Coxiella burnetii strains from brown rats and cattle: are they related?

P2-24] Katelynn Doiron – Coxiella burnetii subversion of the macrophage unfolded protein response

P2-25] Amanda Dragan – Characterizing Coxiella burnetii infection of human lung epithelial cells

P2-26] Nadia Haddad – Follow-up of intra-herd circulation of Anaplasma phagocytophilum strains in cattle farms in France and Germany by molecular typing

P2-27] Claudio Hurtado – Molecular detection of Anaplasma phagocytophilum DNA in thoroughbred horses from Concepcion race course, Concepcion, Chile.

P2-28] Sandra Perez-Macchi – Molecular prevalence of Anaplasma phagocytophilum in domestic dogs from Asuncion, Paraguay

P2-29] Mustapha Dahmani – Molecular investigation and Phylogeny of Anaplasmataceae species infecting animals and ticks in Senegal

P2-30] Katja Strasek Smrdel – Filling the gap on the knowledge of Candidatus Neoehrlichi mikurensis in Europe, Slovenia

P2-31] Ismail Lafri – Detection of a novel relapsing fever Borrelia hispanica, Bartonella spp. And Anaplasmataceae bacteria in Argasid ticks in Algeria.

P2-32] Angela Sweed – Diagnosis of Anaplasma phagocytophilum in the UK

P2-33] Denys Pogoryelov – Chlamydia energy metabolism revisited: V1A-ATPase in focus as a novel drug target

P2-34] Jianmin Zhong – The vitamin B9 supplying capacity of Rickettsia species phytype G021, an endosymbiont in Ixodes pacificus

P2-35] Awa Ido – Pangenome analysis and species delimitation revisited: insight into the Rickettsia genus
P3-1) Mohamed Rahal – First genotyping of Coxiella burnetii from dairy cows in northern Algeria

P3-2) Chien-Chung Chao – Metabolic profiling of Orientia tsutsugamushi infected mouse serum from a chigger-fed mouse model

P3-3) Kristin E. Mullins – Comparative pan-genome analyzes of forty Orientia tsutsugamushi whole genome sequences demonstrate unprecedented gene duplication and divergence

P3-4) Sean Evans – Two Orientia tsutsugamushi effectors mediate NF-kB o65 nuclear export

P3-5) Nam-Hyuk Cho – Diversification of Orientia tsutsugamushi genotypes by intragenic recombination and their potential expansion in endemic areas

P3-6) Ana M. Palomar – Genetic characterization of a potentially new Rickettsia sp. in Rhipicephalus simus from South Africa

P3-7) Khalid El Karkouri – Comparative genomics and proteomics of four spotted fever group Rickettsia spp.

P3-8) Mustapha Dahmani – The isolation of novel Rickettsia-like organism from Cimex lectularius bedbug

P3-9) Tahar Kernif - Common ectoparasites of humans in Algeria : specimen collection and vector-borne agents

P3-10) Algimantas Paulauskas – Detection of spotted fever group (SFG) Rickettsia in Ixodes ricinus ticks from migrating passerine birds

P3-11) Dalyte Mardosaite-Busaitiene – First detection of Rickettsia helvetica in small rodents in Lithuania

P3-12) Sonja E. van Roeden – Increased incidence of non-Hodgkin lymphoma in chronic Q fever patients

P3-13) Halie K. Miller – Coxiella burnetii antibody Seropositivity is not a risk factor for AIDS-related non-Hodgkin lymphoma

P3-14) Vincent Pommier de Santi – Q fever outbreak epidemiologically linked to three-toes sloth, Cayenne, French Guiana, 2013

P3-15) Cléa Melenotte – Acute Q fever endocarditis

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P3-17) Sophie Edouard – Cardiovascular Coxiella burnetii infections in patients presenting with low antibody levels

P3-18) Seon Do Hwang – Molecular detection of Coxiella burnetii in South Korea from two Q fever patients

P3-19) Sonja E. van Roeden – Identification of Coxiella burnetii in non-Hodgkin lymphoma tissue samples
P3-20) Sonja E. van Roeden – High incidence of primary and secondary arterial fistulae during chronic Q fever

P3-21) Raquel Alvarez-Alonso – Genotyping Coxiella burnetii from livestock using SNP and MST techniques

P3-22) Marcella Morì – Genotyping of Coxiella burnetii strains from bovine abortions in Belgium

P3-23) Olga Freylikhman – Whole genome sequencing of four strains of Coxiella burnetii allows estimate host-associated genomic features

P3-24) Stanislav Shpynov – Using of formal order analysis for comparative analyses of Coxiella burnetii genomes

P3-25) Julian Pechstein – The Coxiella burnetii T4SS effector AnkF is important for intracellular replication

P3-26) Aurélien Joulié – qPCR-based determination of the number of IS1111 elements in the genome of C. burnetii strains circulating in France.

P3-27) John P. Prior – development of a novel virus-like particle sub-unit vaccine against Coxiella burnetii

P3-28) Ilse Kouijzer – The value of FDG-PET/CT in diagnosis and during follow-up of 273 patients with chronic Q fever

P3-29) Basma El Hamzaoui – Detection of Bartonella quintana in fleas by MALDI-TOF M

P3-30) Mehdi Boucheikhchoukhi – Ticks (Acari: Ixodidae and Argasidae) of domestic and wild animals and their associated microorganisms in North Eastern of Algeria

P3-31) Rita Abou-Abdallah – Genomic analysis has a key role in the study of intracellular bacteria

P3-32) Bernard Davoust - Molecular detection of Ehrlichia canis in dogs from three districts in Punjab (Pakistan)

P3-33) Nam-Hyuk Cho - Role of type I interferon responses during orientia tsutsugamushi infection

P3-34) Elodie Rousset - Q fever in cattle, sheep and goats: results of a 2012-2015 study in France

P3-35) Yassina Bechah - Liposcelis bostrychophila as a potential source of contamination with Rickettsia felis: a murin animal model.
Philippe Parola – Use the MALDI-TOF MS to identify arthropod vectors of rickettsioses and to detect the microorganisms they carry

For the last four years, our team has developed the use of MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) for arthropod identification. This technology is based on the thorough comparison of protein profiles of the submitted samples with a database of reference spectra, and has revolutionized the field of clinical microbiology. It is now routinely used for the rapid identification of bacteria and fungi from clinical samples. The use of MALDI-TOF MS for the identification of arthropods involves several steps, including i) determination of the body part of the arthropod which will be used for MS analyses (ideally the smallest, to save the remaining body parts for further analyses); ii) construction a database of reference protein profiles of definitely identified specimens; iii) completion of blind tests to check if the specimens are correctly identified when compared to the database; iv) continual updating of the database with new species. We have applied this method to the identification of several hematophagous arthropods, including major vectors of rickettsial diseases. We present here these data as well as promising results for the distinction of arthropods infected or not by rickettsiae.
Many fundamental principles regarding tick-borne rickettsioses in the North America have required re-examination since the beginning of the 21st century. As recently 2002, all tick-borne rickettsiosis in the United States and other continents of the western hemisphere was attributable to infection with a single pathogen, *Rickettsia rickettsii*. Furthermore, *Dermacentor* species ticks were considered the only relevant vectors of this pathogen in the United States. During the next decade a series of discoveries changed paradigms that had existed for the preceding 100 years. These included the recognition that *Rickettsia parkeri* and *Rickettsia philipii* (*Rickettsia* sp. 364D) caused rickettsiosis in the southeastern, southwestern, and western United States. Also during this period, epidemic levels of Rocky Mountain spotted fever emerged among multiple impoverished communities of the southwestern United States and in several states of northern Mexico, caused by explosive and unchecked populations of free-roaming dogs and *R. rickettsii*-infected *Rhipicephalus sanguineus* ticks. Unique species or strains of pathogenic *Rickettsia*, including many that were isolated from ticks during preceding decades but remained uncharacterized until the advent of molecular techniques, have now been characterized and are likely to influence the epidemiology of spotted fever group rickettsiosis in various regions of the United States. Included among these is *R. parkeri* strain Black Gap, associated with *Dermacentor parumapertus* ticks in the western United States and nearly identical to *R. parkeri* strain Atlantic rainforest, a pathogenic species likely responsible for hundreds of cases of a non-lethal spotted fever group rickettsiosis in Brazil. Another is *Rickettsia* sp. Tillamook, a pathogenic *Rickettsia* species associated with the human-biting tick *Ixodes pacificus* in the western United States that is related only distantly to other *Rickettsia* of the spotted fever group. Collectively, these processes emphasize the dynamic nature of tick-borne rickettsioses, and the necessity to continuously consider the fluid and varied ecological, social, and temporal interactions among humans, ticks, vertebrate hosts and *Rickettsia* in the emergence and epidemiology of these diseases.
Gilbert Greub – New insights in the biology of *Chlamydia*-related bacteria

During the last decade, a number of novel chlamydia-related bacteria have been isolated that largely enlarged our vision of the bacterial biodiversity within the Chlamydiales order. Moreover, thank to the recent technological advances in genomics and the availability of a number of genomes, we gained insight in the evolution of Chlamydiales and some specific features have been discovered, revealing the fascinating history of intracellular parasitism of these strict intracellular bacteria. Furthermore, during last years, the availability to transform Chlamydiales open new perspectives to precise the function of specific proteins, either shared by all Chlamydiales or specific only to a given species, and that include among others several virulence factors. The virulome of Chlamydiales is indeed relatively large including adhesins and effectors of the type III secretion system that helps Chlamydiales to corrupt the host cell. Thank to genomics coupled with molecular biology, it was also possible to decipher the mechanisms involved in chlamydial division, in the maturation of elementary bodies into replicative reticulate bodies, and in the persistence of these strict intracellular bacteria as aberrant bodies, when exposed to stressful conditions. Altogether, during this last decade, a number of discoveries have been made often thank to the study of chlamydia-related bacteria, that exhibits some specific characteristics that help better understanding the Chlamydiales order as a whole.
Chlamydiaceae feature a biphasic life cycle and cause acute and chronic diseases. The obligate intracellular pathogens do not need to resist osmotic challenges inside their host-derived vacuole and thus lack a cell wall. Nevertheless, they synthesize peptidoglycan to support an unusual process of cell division. The genomically-reduced Chlamydiaceae lack the cell division organizer FtsZ and instead use the actin homolog MreB. Cell division is initiated by a budding mechanism and the asymmetric cell poles mature into two equally sized daughter cells separated by a septum which contains a peptidoglycan ring. The transient ring is remodelled during constriction and degraded afterwards. Moreover, β-lactams do not kill Chlamydiaceae but block cell division and induce reversible persistence. The underlying mechanisms of this phenomenon are not fully understood.

We explored the cellular function of the peptidoglycan biosynthesis pathway in Chlamydiaceae and linked evolutionary conservation of peptidoglycan precursor lipid II cycling to cell division. AmiA, the chlamydial homolog of a PG hydrolase which is in E. coli important for cell separation, is a novel penicillin target. Contrary to free-living bacteria, the chlamydial AmiA uses the ultimate peptidoglycan precursor lipid II as a substrate and has dual activity as amidase and carboxypeptidase. The latter function is sensitive to penicillin and assigned to a motif that is usually found in penicillin-binding proteins (PBPs). Moreover, the peptidoglycan binding protein CPn0902 serves as a novel carboxypeptidase and chlamydial PBP6 as a penicillin-sensitive carboxypeptidase. In addition to these findings, a summary on our recent research relating to the lipid II processing and peptidoglycan-ring remodelling machinery including PBPs will be given.

In total, Chlamydiaceae may emerge as new model systems to understand how a minimal and modified cell wall biosynthesis machinery supports FtsZ-independent prokaryotic cell division.
Recently, organisms resembling *Chlamydiae* have been detected in the environment (soil, water), amoebae and various animals, including mammals, reptiles, arthropods, isopods and fish. These organisms are called **Chlamydia-like bacteria**. They share intracellular lifestyle, biphasic developmental cycle and a large core gene set (the "Pan-Genome of the Chlamydiae") with the genus *Chlamydia*. The chlamydia-related bacteria include eight family-level lineages of genetically related bacteria including *Parachlamydiaceae*, *Waddliaceae*, *Simkaniaeae*, *Rhabdochlamydiaceae*, *Criblamydiaceae*, *Piscichlamydiaceae*, *Clavichlamydiaceae* and *Parilichlamydiaceae*.

Some of these recently identified chlamydia-related bacteria may have human pathogenic potential: *Waddlia chondrophila* is associated with tubal factor infertility, adverse pregnancy outcome, and lower respiratory tract infection, and *Simkania negevensis* and *Rhabdochlamydia* spp. are found in association with respiratory infections. The role of *Parachlamydia acanthamoebae* as an agent of pneumonia is suggested by seroepidemiological and molecular studies as well as by establishment of a murine pneumonia model. DNA of various chlamydia-related bacteria has been detected in skin with and without any pathology. The transmission routes have remained enigmatic, but ticks can represent a transmitting vector.
Among members of the Chlamydiales order, several bacterial families are recognized as pathogenic for humans and animals. However, vectors and reservoirs of these chlamydiae are poorly described and remain to be better characterized. Arthropods, and particularly ticks, are known to be vectors of several intracellular bacteria, and Chlamydiales DNA was recovered from tick samples in several recent studies. To go further in the potential role of tick as vectors of chlamydiae, we have investigated the ability of chlamydiae to infect and grow within tick cell lines.

We have infected two tick cell lines, from hard ticks, with several species representative of a broad spectrum of families. Bacterial growth was assessed by qPCR and immunofluorescence microscopy. Recognized human pathogen C. trachomatis was able to enter cells, and despite its incapacity to replicate efficiently, the bacteria were able to persist within cells. It was also the case for P. acanthamoebae. However, despite the low temperature growth (28°C) of cells, W. chondrophila and E. lausannensis were able to enter and grow efficiently, inducing a lysis of tick cells 48 hours post-infection. Interestingly, in only one cell line, we could observe the presence of aberrant bodies, the persistent form of chlamydiae. These results suggest ticks as new hosts for chlamydiae, and highlight the need to further investigations on the role of ticks as reservoirs and vectors of these bacteria.
Delaney Burnard – Novel Candidatus Rhabdochlamydia sp. associated with both native Australian marsupials and their parasiting tick species

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Members of the order Chlamydiales are known for both their potential as human and veterinary bacterial pathogens. The ever expanding species diversity and host range of the Chlamydiales leaves epidemiological factors such as routes of transmission and pathogenesis for many of its species undefined. Ticks are well known vectors for many infections, with several European reports recently describing the presence of the Chlamydiales in these arthropods. Native Australian marsupials are hosts to an extensive range of tick species, however little is known about the relationship between marsupials, their parasitising tick species and the Chlamydiales. Using a Chlamydiales PCR screening approach we’ve screened 438 ixodid wildlife parasitising ticks and over 250 individual marsupials from both sympatric and allopatric locations across Australia for the presence of chlamydial DNA. Chlamydiales abundance, diversity and host range was vast and unexpected, with one previously described species and 12 novel Chlamydiales genotypes representative of novel genera and species identified. Interestingly, one novel Ca. Rhabdochlamydia porcellionis genotype was identified in both ticks, koalas and non-koala marsupials from the same geographic regions. Comparative genomics investigations of this novel chlamydial species from both marsupials and ticks are currently underway to provide further information into the transmission and potential pathogenesis of this Chlamydiae.
Rapid progresses in diagnostic techniques have enabled the discovery of several novel Chlamydia-related bacteria, including *Simkania negevensis*. Mostly known for the pathogenic *Chlamydia* spp., the *Chlamydiaceae* order is now composed of at least 9 family-level lineages, each harboring specific biological characteristics. *S. negevensis* represents the founding member of the *Simkaniaceae* family. Discovered in 1992, it is suspected to be associated with respiratory diseases, at least in the Middle East, and could represent an emerging pathogen. We evaluated the antimicrobial susceptibility patterns of this emerging bacterium towards twelve antibiotics from 8 different classes using a quantitative approach and highlighted significant differences with other related *Chlamydiaceae*. *S. negevensis* was susceptible to macrolides, clindamycin, cyclines, rifampicin and quinolones. Importantly, unlike other *Chlamydiaceae*, treatment with β-lactams and vancomycin did not induce the formation of aberrant bodies leading to a complete resistant phenotype. On the other hand, a small fraction of *S. negevensis* particles showed abnormal morphological aspects under phosphomycin treatment. The varying responses of *S. negevensis* to different cell-wall inhibitors, each targeting a specific step of the peptidoglycan biosynthesis, supports that some significant differences exist in the peptidoglycan biosynthesis pathway among *Chlamydiaceae*. This may bring further insights into the mechanisms of *Chlamydiaceae* cell division.
Human monocytotropic ehrlichiosis is an emerging, life-threatening, tick borne zoonosis that is caused by obligately intracellular bacterium, *Ehrlichia chaffeensis*. *E. chaffeensis* infects mononuclear phagocytes and replicates within membrane bound cytoplasmic vacuoles. The mechanisms whereby *E. chaffeensis* survives and evades innate host defense mechanisms of these primary innate immune cells have remained elusive. Recently, we have identified and molecularly characterized a group of type 1 secreted tandem repeat protein (TRP) effectors, and defined a diverse group of TRP effector-host interactions. These newly defined molecular effector-host interactions reveal the large breadth and complexity of TRP-host interactions, and the many host cell processes influenced by these effectors, including but not limited to cell signaling, transcriptional regulation, post translational modifications, and apoptosis. Specific host cell pathways that are targeted by ehrlichial effectors are notable and include the evolutionarily conserved eukaryotic signaling pathways Wnt and Notch. Recently, we have demonstrated that *E. chaffeensis* TRP120 is a moonlighting effector that functions as a nucleomodulin that directly modulates host cell gene transcription, but also directly activates Wnt and Notch pathways. Wnt and Notch signaling control several innate defense mechanisms including pattern recognition receptor expression, phagocytosis, autophagy, and proinflammatory cytokine production. Studies in our laboratory are focused on revealing how ehrlichiae exploit these host cell pathways to influence innate immune defense mechanisms and promote ehrlichial survival in the mononuclear phagocyte.
Inflammasomes are cytosolic multiprotein complexes triggered in response to diverse stimuli, including lipopolysaccharide (LPS), to induce caspase-1 activation and IL-1beta secretion. Ehrlichia, the causative agent of human monocytic ehrlichiosis (HME), is an obligate intracellular Gram-negative bacterium that lacks LPS but causes liver injury and fatal toxic shock, secondary to inflammasome activation. However, the molecular mechanism leading to development of detrimental inflammasome activation remains elusive. We demonstrate here that MyD88 signalling is a major host-pathogenic pathway inducing inflammasome activation during fatal ehrlichiosis. MyD88-deficient (MyD88/-) mice exhibited significantly attenuated liver injury and survived significantly longer than wild type (WT) mice following lethal infection, despite increased bacterial burden in liver. Enhanced resistance of MyD88/- mice to fatal ehrlichiosis correlated with attenuated inflammasome activation, shown by reduced caspase-1 activation/cleavage by Western blot, and decreased serum level of IL-1beta. Mechanistically, we found MyD88 elicits inflammasome activation in primary macrophages via activation of the mammalian target of rapamycin complex 1 (mTORC1), subsequent inhibition of autophagic flux and reduced mitochondrial autophagy (mitophagy). Blocking mTORC1 signaling in vivo and in in vitro-infected macrophages enhanced autophagy and attenuated inflammasome activation, but also promoted bacterial replication, which may account for the discrepancy between attenuated immunopathology and bacterial burden in MyD88/- mice. Together, this study reveals a novel regulatory pathway of host-pathogenic inflammasome activation during fatal ehrlichiosis via MyD88-dependent mTORC1 activation and inhibition of macrophage mitophagy. However, as Ehrlichiae exploit autophagy to obtain nutrients for growth our data suggest MyD88 signaling also functions as a host-protective mechanism by preventing autophagy induction and intracellular bacterial survival within phagocytic target cells.
Anaplasmataceae is a family of α-proteobacteria that belongs to the order Rickettsiales and includes the genera Anaplasma, Ehrlichia, Neorickettsia and Wolbachia. From these genera, Ehrlichia and Anaplasma are important pathogens affecting animals and humans. Ehrlichia are obligate intracellular gram-negative, tick-borne bacteria that grow within membrane-bound vacuoles in human and animal leukocytes causing ehrlichiosis. Since the reorganization of the family Anaplasmataceae in 2001, only five species of Ehrlichia spp. are officially recognized (i.e. E. canis, E. chaffeensis, E. ewingii, E. ruminantium and E. muris). In 2016, a novel Ehrlichia species was accepted by the International Committee on Systematics of Prokaryotes as E. minasensis. At the ultrastructural level, this novel bacteria resembles E. canis, E. chaffeensis, and E. muris. Full genome sequencing and phylogenetic analysis using several bacterial genes showed that E. minasensis is closely related to E. canis. Interestingly, E. minasensis was initially isolated from Rhipicephalus microplus ticks, while E. canis is mainly found in R. sanguineus. Independently, another group in Brazil reported E. minasensis to be pathogenic for cattle, while E. canis is mainly pathogenic for dogs. Further evolutionary analysis using the major glycoprotein TRP36 suggested that E. minasensis evolved recently from a highly divergent and variable clade within E. canis. The emergence of new pathogens is frequently associated to mutations that confer the ability to infect novel hosts, known as “host shift”. Comparative genome analysis between E. canis and E. minasensis might shed light on the genetic basis that allowed E. minasensis to colonize a new set of vertebrate and invertebrate hosts.
Ehrlichia chaffeensis infects mononuclear phagocytes and survives intracellularly by exploiting host cell processes to evade host defenses. The mechanisms involved are not fully defined, but appear to rely largely on a subset of tandem repeat proteins (TRP) effectors. E. chaffeensis TRPs are type 1 secreted effectors that interact with a functionally diverse group of host cell targets associated with various biological processes. In this study, we investigated the influence of TRP host target proteins on ehrlichial infection by RNA interference. In total, 138 TRP-interacting host proteins identified by yeast two-hybrid were targeted by siRNA and the infection level determined by real-time qPCR. Knockdown of 124 (89%) TRP target proteins had significant influence on infection either by inhibiting (85%) or promoting (15%) ehrlichial infection. Notably, knockdown of 18 host proteins which interacted with TRP120 promoted the infection, suggesting that these targets may be degraded to promote infection. Host proteins that interact with TRPs are involved in cellular processes, including cell signaling, vesicle trafficking and intracellular transport, transcriptional regulation, metabolism, protein posttranslational modification, and apoptosis. Selected host targets were examined by immunofluorescent microscopy during infection and were found to localize with the morulae, or in the host cell cytoplasm adjacent to morulae. This study confirms that the majority of host proteins known to interact with TRP effectors influence infection and further extends the current knowledge that E. chaffeensis TRPs participate in a complex array of host protein interactions in order to reprogram the host cell and promote intracellular.
Ehrlichia chaffeensis is a gram negative, obligately intracellular pathogen that causes the emerging tick borne zoonosis, human monocytotropic ehrlichiosis. Antibodies are essential for clearance and protection against E. chaffeensis, and previous studies have determined that Fc receptors are involved. Antibodies to OMP-1 and TRPs provide protection against infection in vivo, and epitope-specific antibodies against TRPs demonstrated protection against E. chaffeensis in vitro when administered both prophylactically and therapeutically, suggesting existence of both extracellular and intracellular antibody-mediated immune mechanisms. Thus the mechanisms and cellular context of antibody mediated immunity to E. chaffeensis are not completely defined. We investigated the capacity of several E. chaffeensis-specific human monoclonal antibodies (huMAbs) in mediating bacterial clearance, using an in vitro model of cellular infection. Anti-OMP-1 HuMAbs capable of restricting E. chaffeensis infection were further evaluated to determine the cellular context and molecular mechanisms involved in antibody-mediated clearance. Extracellular blocking of ehrlichial entry was observed with one huMAb, as evidenced by significant reduction of ehrlichial entry into cells and reduced infection. Another huMAb did not block entry, but was carried into the cell complexed with ehrlichiae. Intracellularly it was recognized by the high affinity cytosolic Fc receptor TRIM21, and activated innate immune signaling pathways, thereby reducing infection. The intracellular antibody-mediated immune signaling and inhibition was significantly abrogated by genetic deletion of TRIM21. For the first time, we have demonstrated that antibodies targeting a single major outer membrane protein provide protection through two distinct mechanisms including blocking entry, and intracellularly by the recruitment of TRIM21.
Sequential expression of outer membrane protein antigenic variants is an evolutionarily convergent mechanism used by bacterial pathogens to escape host immune clearance and establish persistent infection. Variants must be sufficiently structurally distinct to escape existing immune effectors yet retain core structural elements required for localization and function within the outer membrane. We examined this balance using Anaplasma marginale, which generates antigenic variants in the outer membrane protein Msp2 using gene conversion. The overwhelming majority of Msp2 variants expressed during long-term persistent infection are mosaics, derived by recombination of oligonucleotide segments from multiple alleles to form unique hypervariable regions (HVR). As a result, the mosaics are not under long-term selective pressure to encode a functional protein; consequently, we hypothesized that the Msp2 HVR is structurally permissive for mosaic expression. Using an integrated approach of predictive modeling with determination of native Msp2 protein structure and function, we demonstrate that structured elements, most notably β-sheets, are significantly concentrated in the highly conserved N- and C-terminal domains. In contrast the HVR is overwhelmingly random coil with the structured α-helices and β-sheets confined to the genomically defined "structural tethers" that separate the antigenically variable microdomains. This structure is supported by the surface exposure of the HVR microdomains and the slow diffusion type porin function in native Msp2. Importantly, the predominance of random coil provides plasticity for formation of functional HVR mosaics and realization of the full potential of segmental gene conversion to dramatically expand the variant repertoire.
Anne-Claire Lagrée – Exploration by a dual approach of the role in cattle abortion of an *Anaplasma phagocytophilum* vacuolar membrane protein

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*Anaplasma phagocytophilum* is a zoonotic tick-borne obligate intracellular pathogen. This bacterium can infect various mammalian species and humans. The disease in domestic ruminants is characterized by diverse signs including abortions, and leading to significant economic losses. However, in France, the potential frequency of *A. phagocytophilum*-related abortions is unknown, and thus, it is proving to be difficult to estimate its overall economic impact. This is partly explained by the absence of suitable and specific tools capable of detecting *A. phagocytophilum* associated with abortion. Our first objective was to identify a genetic marker able to distinguish between *A. phagocytophilum* strains isolated from domestic ruminants that had aborted and those that had not. We typed a total of 123 *A. phagocytophilum* isolates obtained from cattle, including 25 from cows that had aborted, via multiple-locus variable-number tandem repeat (VNTR) analysis. A multivariate logistic model demonstrated that the triple-repeat allele of the APV-A VNTR was statistically more frequent in *A. phagocytophilum* from cattle that had aborted, compared to *A. phagocytophilum* from cattle that had not aborted (*P* = 0.03), while controlling for any regional effects (*P* < 0.0001). The APV-A triple-repeat VNTR allele could thus be used as a marker of *A. phagocytophilum* involved in abortions. Interestingly, this VNTR is located inside an *A. phagocytophilum* occupied vacuolar membrane protein, APH_0032. Due to its location, this protein is considered as a critical host-pathogen interface. We hypothesized that APH_0032 is an effector protein that interacts with multiple host cell proteins that are essential for cellular entry and survival. In a second step, using yeast two-hybrid analysis with a bovine cDNA library, we found one host protein, NACA (nascent polypeptide-associated complex alpha subunit), that interacted with APH_0032 from a bovine isolate with 1,5 repeats of APV-A, as it had already been sequenced in our laboratory. This interaction, obtained three times, was confirmed by coprecipitation in HEK-293T cells. Very few publications on the interactions between NACA and proteins of pathogens are available to date. Functional analyses are needed to validate our results. Moreover, we now need to determine if APH_0032 with triple repeat APV-A is also capable to interact with this host protein, or links to other interactors from the host cell. This could lead to explain why the APV-A triple repeat VNTR allele is preferentially involved in abortions.
Bartonella spp. are Vector Borne bacteria. Among them, B. henselae induces cat scratch disease, one of the most frequent urban zoonoses. The cat is its main reservoir. Initial typing studies of strains isolated from Human and cat have underlined a discrepancy between the frequency of the types of stains isolated from cats and the frequency of the types strains infecting human. A more discriminating technique using Multi Locus VNTR Analysis demonstrated that feline hosts are infected by two different groups of strains: Group A strains almost only isolated from cats and group B strains found in cats but also in dog and human.

In order to elucidate potential mechanisms of transmission from cat to human supported by group B strains, sequencing of the genome of ten feline strains belonging to the both group have been realized. Analysis of these genomes confirms the reality of these two groups. The comparison of genomes led to identify differences characterized by deletions in the genome of Group A strains. Some of these deletions affect genes which could be involved in the mechanism of transmission to human by cat scratch. One of these gene codes for an outer membrane protein (Arp protein) that seems to be expressed by Group B strains but not by group A strains.
Bruno Chomel – *Bartonella*: from cats to bats

Bruno B. Chomel, DVM, PhD; Professor of Zoonoses, School of Veterinary Medicine, university of California, Davis, USA

Bartonella are gram negative bacteria, mainly vector-borne and targeting red blood and endothelial cells. Since the discovery of *Bartonella henselae* as the agent of cat scratch disease, many new species or subspecies have been described in mammals, including humans, and also reptiles and birds. Domestic cats are the main reservoir for *B. henselae* and *B. clarridgeiae*. A wide range of *Bartonella* species have also been described in domestic dogs, wild canids and ruminants. However, the most diverse reservoirs of bartonellae are rodents and bats. This presentation will focus on recent information on feline and canine infections and on work performed on Mexican bats, especially vampire bats.
Aranzazu Portillo – Prevalence (culture, PCR and serology) of *Bartonella* spp. in asymptomatic people working in a sanitary center from La Rioja, Spain

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*Bartonella* spp. are fastidious Gram-negative bacilli classified within the class Alphaproteobacteria. They are mainly transmitted by arthropod vectors and/or direct contact with animals. Certain species are recognized human pathogens (e.g. *Bartonella henselae*), while others have been occasionally described as causative agents of disease (e.g. *Bartonella rochalimae*). Microbiological culture of *Bartonella* is slow and rarely successful. Molecular tools allow the quick detection of *Bartonella* DNA in certain clinical samples, but sensitivity is not optimal and techniques are not routinely available. Serological assays are usually the choice for diagnosis, despite cross reactions and delay for diagnosis since a convalescent specimen is needed. In the last decade, a method for diagnosis of *Bartonella* infections is incorporating to the study of animal and human diagnostic specimens. It is based on an enrichment growth medium for *Bartonella* culture that improves the yield of *Bartonella* species detection by subsequent molecular biology techniques. In the context of an agreement among North Carolina State University, Galaxy Diagnostics Inc. and the Center for Biomedical Research from La Rioja (CIBIR) for the study of zoonoses under the perspective of ‘One Health’, our aim was to investigate the prevalence of *Bartonella* spp. in asymptomatic people working in a sanitary centre. EDTA-blood and sera samples from 100 health workers (doctors, nurses, students and research staff) from Hospital San Pedro-CIBIR (Logroño, Spain) were obtained in May-June 2016. Culture of samples in the enrichment *Bartonella*-Alpha-Proteobacteria-Growth Medium (BAPGM) and subsequent PCR (16S-23S rRNA of *Bartonella*) and sequencing were carried out. Indirect immunofluorescent assays using *Bartonella* spp. antigens were performed. Participants completed a standardized questionnaire. Results will be presented and potential associations between microbiological and epidemiological findings will be discussed. Acknowledgements: Bayer Animal Health GmbH, Germany.
Richard Hassall – Understanding the influence of host demography on *Bartonella* diversity

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The ability of a pathogen to adapt to changing conditions and compete will be dependent on genetic diversity. In the case of bacteria, genetic diversity can be generated through mechanisms such as horizontal gene transfer, and the spatial structure of populations, which will rely on the spatial structuring of hosts. However, we still have a limited understanding of the ecology and evolution of bacteria in natural host populations. Dispersal of hosts between populations and the dynamics of host within a population may play a role in influencing the genetic diversity of bacteria by creating opportunities for genetic exchange between individuals. Therefore, the spatial structuring of a host population could be crucial in influencing both the ability of pathogens to disperse and the generation of new diversity, particularly in fragmented populations.

Here we use microparasites from the genus Bartonella as a model system to determine how the spatial dynamics of host populations may influence the genetic diversity of pathogens in natural reservoirs. We focus on Bartonella spp. infecting two contrasting water vole populations. A metapopulation, where small subpopulations are linked by frequent dispersal events, and a network of island populations, where populations are generally larger but dispersal events between islands are rare. Using multilocus sequence typing, we quantify and compare the genetic diversity of Bartonella spp. infecting these two host systems. By investigating the relationship between host demography and the standing genetic variation of microparasites we can begin to tease apart the ecological processes that maintain pathogen diversity in host populations.
Adam Polkinghorne – Genomic analyses provide insight into the evolution and adaptation of intracellular bacteria within phylum *Chlamydiae*

Adam Polkinghorne

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The phylum *Chlamydiae* is comprised of an increasingly diverse but monophyletic group of bacteria that share a unique biphasic developmental cycle. The most well described members belong to the family *Chlamydiaceae*, home to a range of important human and animal-borne pathogens. The advent of new molecular and culture-based tools has also revealed a growing number of new families and member species from host-associated and environmental sources. As with most other pathogens, genomics has revolutionised our understanding of the biology, pathogenicity and evolution of these organisms. While initial genomic studies (i) faced a significant bottleneck associated with the need to laboriously culture strains to obtain sufficient chlamydial DNA for sequencing and; (ii) biased analysis to those strains which could be cultivated, several recent culture-independent genomic methods have emerged that enable researchers to access the genomes of previously described chlamydiae from clinical samples as well as novel chlamydiae. The resulting comparative genomic studies have revealed new insights into the evolution and adaptation of bacteria in the phylum *Chlamydiae*, including (i) the identification of a series of new chlamydial pathogens belonging to existing and novel chlamydial families; (ii) the early acquisition of chlamydial virulence mechanisms that are required for manipulation of the host cell environment; (iii) contrasting epidemiological pictures for chlamydial pathogens that occupy the same host niche; and (iv) evidence for genomic reduction and genomic expansion in relation to the predicted host range of the chlamydial species.
Chlamydia have two extrachromosomal elements - a small single-stranded DNA microvirus (chlamydiaphage) with a 3,200nt circular genome and plasmid of 7,500 bp which encodes eight genes. The recent development of a plasmid-based transformation protocol for Chlamydia species in our laboratory has led to renewed interest in the role and function of the plasmid and its individual genes and in the use of the chlamydiaphage as a vector. The study of natural variants, sequence comparisons and phylogenetic analyses has revealed insights into plasmid gene function. These observations coupled to detailed gene deletion studies have guided improvements in vector development. Despite these significant advances we still know very little about gene regulation in the plasmid. The plasmid in *C. trachomatis* can naturally transfer between strains although there appear to be significant barriers to plasmid transfer between chlamydial species. Plasmid recombination and evidence that the plasmid has a role in virulence will be discussed.
Chlamydia spp. represent elegantly complex pathogens, yet definitively elucidating the function and role of chlamydial gene products has been historically complicated by an inability to genetically manipulate chlamydiae. This has all changed over six short years. The advent of a tractable method for transformation of chlamydiae, in particular, has radically altered how chlamydial pathogenesis is investigated. Transformation and inducible gene expression coupled with random and targeted mutagenesis techniques have now provided initial in-roads for definitive characterization of chlamydial infection biology. Mutagenesis was initially employed, in a confirmatory fashion, to test functional activities deduced during the pre-genetic era. Discovery of new, Chlamydia-specific biology represents the most exciting prospect for future progress and examples are beginning to emerge. Despite these important advances, the chlamydial genetic tractability quotient remains low and there is a need for further development. Increased transformation efficiency and capabilities such as deletion of essential genes, marker-less gene inactivation, and efficient genetic manipulation of other chlamydial species represent further requirements to complete an efficacious molecular toolbox. The current state-of-the-art in chlamydial genetics will be discussed along with a forward-looking consideration of how alternatives, potential pitfalls, and areas of focus are likely to shape our understanding of the chlamydial approach to obligate intracellular parasitism. Forward and Reverse genetic approaches are now available for genetic manipulation of the chlamydiae.
All known Chlamydiae are obligate intracellular bacteria exhibiting a biphasic life cycle. The phenotypic coherence of this phylum is rarely observed among bacteria. The difficulty to cultivate Chlamydiae and the fact that genetic manipulations are impossible for most of them drastically slow down the study of their biology. Most efforts concentrate on Chlamydia trachomatis, an important human pathogen. Nevertheless, an increasing number of genomes of new chlamydial species are being sequenced. The goal of this project was to build a genome database allowing comparisons of chlamydial genomes and facilitating annotation transfers from well-studied genomes, and to provide a framework for community-based manual update of the annotation of all chlamydial genomes. The database includes representative genomes of the Chlamydiaceae and all sequenced Chlamydia-related strains. The annotation of all genomes was completed in a consistent manner using KEGG, interproscan, Uniprot, COG and operon information. Type III secretion system effector repertoires were predicted and compared at the scale of the whole phylum. We also intend to provide information about genome-scale experimental data. All entries are cross referenced with widely used databases such as Uniprot and Genbank. Proteins were clustered into orthologous groups, allowing pairwise and multiple genome comparisons. Multiple sequence alignments and phylogenies were reconstructed for each orthologous groups. That information may help researchers to identify core chlamydial genes involved in their unique intracellular lifestyle. The purpose of the database is to provide a high quality resource for the chlamydial research community.
Loyd Vaughan – *Chlamydia*-related bacteria and related epitheliocystis in fish

The *Chlamydiae*, dominant intracellular pathogens of land animals, including humans, and comprising an estimated 180 families, are marine in origin. *Chlamydia* commonly infect the gills (epitheliocystis), leading to distress and mortalities, especially of young fish. A global problem, fish chlamydial agents have been described in Australian, Chilean, North American and European aquaculture, in fresh water carp from Africa and in salmonids from Swiss rivers and fish farms. In a marine setting, antibiotic treatments are often ineffective. There are no vaccines for these fish pathogens and as none of these agents could be brought into culture, we are still a long way from developing them.

The focus has been on developing tools for a thorough characterization of of epitheliocystis agents directly from tissues, including detailed genomic and morphological analysis. Chlamydial agents infecting fish belong to the deepest rooted phylogenetic clades and as such offer a potential glimpse into the evolutionary origins of this phylum. Together with other groups, we could also show that *Chlamydia* are not the sole causes for the disease epitheliocystis, and in this process have discovered a range of novel obligate and facultative intracellular bacteria affecting wild and cultured fish. We have also established a zebrafish model to explore chlamydial infections using *Waddlia chondrophila*.

Lesions similar to epitheliocystis have also been observed in invertebrates and the implications of this finding, and the utility of fish as a source and model for investigations will be discussed.
Chlamydia trachomatis (Ct) has evolved strategies to redirect the host’s signaling and resources for its own survival and propagation. Despite the clinical notoriety of Ct infections, the molecular interactions between Ct and its host cell proteins remain elusive. In this study, we focused on the involvement of the host cell epidermal growth factor receptor (EGFR) in Ct attachment and development. A combination of molecular approaches, pharmacological agents and a range of cell lines were used to demonstrate distinct functional requirements of EGFR versus PDGFRβ in Ct infection. We show that Ct infection increases the phosphorylation of EGFR and of its downstream effectors PLCγ1, Akt and STAT5. While both EGFR and PDGFRβ are partially involved in bacterial attachment to the host cell surface, it is only the knockdown of EGFR and not PDGFRβ that affects the formation of Ct inclusions in the host cells. Inhibition of EGFR results in small immature inclusions, and prevents Ct-induced intracellular calcium mobilization and the assembly of the characteristic F-actin ring at the inclusion periphery. By using complementary approaches, we demonstrate that the coordinated regulation of both calcium mobilization and F-actin assembly by EGFR are necessary for maturation of chlamydial inclusion within the host cell. A particularly important finding of this study is the co-localization of EGFR with the F-actin at the periphery of Ct inclusion where it may function to nucleate the assembly of signaling protein complexes for cytoskeletal remodeling required for Ct development. Cumulatively, the data reported here connect the function of EGFR to Ct attachment and development in the host cells, and this has led to new venues for targeting Ct infections and Ct-associated diseases.
Chlamydia trachomatis delivers multiple Type 3 secreted effector proteins to host epithelial cells to manipulate cytoskeletal functions, membrane dynamics and signaling pathways. In this presentation, I will provide two examples of how emerging forward and reverse genetic tools are enabling a molecular dissection of the function of these various effectors. In the first example, we provide evidence that the effector TepP/Ct875 is important for bacterial replication in cervical epithelial cells, the early activation of Type I IFN genes, and the recruitment of Class I phosphoinositide 3 kinases (PI3K) and the signaling adaptor protein CrkL to nascent pathogen-containing vacuoles (inclusions). The translocation of TepP correlated with an increase in the intracellular pools of phosphoinositide 3,4,5 triphosphate but not the activation of the pro-survival kinase Akt, suggesting that TepP-mediated activation of PI3K is spatially restricted to early inclusions. In the second example, we identify an effector that protects Chlamydia infected cells from potent cell-death defense mechanisms. We screened a library of chemically-generated Chlamydia mutants and identified a strain that induces rapid apoptotic and necrotic death in infected cells. We linked this phenotype to the inactivation of the inclusion membrane protein CpoS/Ct229. Coinfection experiments revealed that the protection from cell death afforded by CpoS is limited to the inclusion in which it resides indicating that this bacterial effector counteracts a spatially restricted pro-death signal. In infected cells, CpoS-deficient Chlamydia induced an exacerbated type I interferon response that was blocked by pharmacological or genetic disruption of the host cyclic-di-nucleotides sensing and activation pathway (cGAS/STING/TBK1/IRF3). However, interfering with this pathway did not prevent cell death, except for genetic disruption of STING, suggesting that this sensor performs separate functions as a regulator of cytokine responses and cell death. CpoS-deficient strains are attenuated in their ability to propagate in cell culture and are cleared faster from the murine genital tract, highlighting the importance of CpoS for Chlamydia pathogenesis.
**Waddlia chondrophila** is a *Chlamydia*-related bacterium that has been implicated as a putative bovine abortifacient. Recently, *Waddlia* has also been implicated in cases of human miscarriage. While the organism’s isolation from bovine abortion material and indirect serological evidence suggests a role in bovine abortion, given the lack of direct evidence, this study was performed to investigate whether experimental challenge of pregnant cattle with *W. chondrophila* would result in abortion and recovery of the organism. Nine pregnant Holstein-Friesian heifers received $2 \times 10^8$ IFU *W. chondrophila* by intravenous challenge at 3 months gestation. Four pregnant negative control animals underwent mock challenge. Rectal temperatures and bloods were obtained at 4 hourly intervals 24 hrs postchallenge. Two negative control and four *W. chondrophila* challenged animals were necropsied two weeks prior to parturition to allow the aseptic collection of tissues for molecular analyses and recovery of the organism. The remaining animals were allowed to proceed to parturition where placentas were collected and dam and calf were necropsied within 48hrs, for collection of samples. Gross examination revealed only a single dam, which was euthanised two weeks prior to parturition, to exhibit large amounts of yellow, semi-solid exudate on the surface of the chorioallantois in the intercotyledonary zones which occasionally extended onto the outersurface of the placentome. Presence of *W. chondrophila* antigen was confirmed by IHC in the placentomes, and foetal tissues were also positive by real-time PCR. There was no evidence of the organism in any of the other challenged or negative control animals. *W. chondrophila* was recovered from the single positive animal and DNA genome sequencing matched that of the challenge strain. The current study demonstrated the presence of *W. chondrophila* DNA in the placenta, amniotic fluids and foetus in a near-term heifer, and critically, viable organisms in the placenta, 6 months after intravenous infection. This study therefore demonstrates the disease causing potential of this organism.
Nicolas Jacquier – An homologue of a sporulation protein of *B. subtilis* is involved in peptidoglycan remodeling during chlamydial division

Nicolas Jacquier, Akhilesh K. Yadav, Patrick H. Viollier, Felipe Cava and Gilbert Greub

All Chlamydiales share a common division mechanism, which is independent of the bacterial division organizer FtsZ, its encoding-gene being absent from all the chlamydial genomes sequenced up to date. Nevertheless, Chlamydiales divide by binary fission, similarly to bacteria possessing FtsZ. Using the Chlamydia-related bacterium *Waddlia chondrophila* as a model, we recently proposed that actin homologue MreB and its regulator RodZ play an essential role in cell division of *W. chondrophila*, in a PG-dependent process. We could also show that the Pal-Tol complex involved in the maintenance of the link between the inner and the outer membrane in Gram negative bacteria is conserved in Chlamydiales and is highly enriched at the division septum. To better understand the relationship between septum formation by RodZ and MreB and peptidoglycan synthesis and remodeling, we initiated screens to find potential interactors of both RodZ and peptidoglycan. Using immunoprecipitation and mass spectrometry analysis, we could identify candidate coordinators of septum formation and peptidoglycan remodeling, among which the homologue of the SpoIID protein, involved in PG remodeling during sporulation in *Bacillus subtilis*. We could show that this protein: (i) localizes to the division septum, (ii) causes division defect when overexpressed in *E. coli*, (iii) directly binds peptidoglycan and (iv) can digest denuded glycan strains in vitro, similarly to its homologue of *B. subtilis*. Our results indicate that Chlamydiales did conserve an homologue of the SpoIID protein during evolution and apparently use it as a specific septal PG-modifying enzyme that allows PG remodeling during division.
Silke Wölfel – First detection of *Rickettsia monacensis* in Denmark

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In Denmark, only evidence of the occurrence of *R. helvetica* in a few regions has been reported, so far. In this study, 7510 ixodid ticks (723 (m)ales, 681 (f)emales, 6106 (n)ymphs, 0 larvae) in 973 pools up to 12 individuals were collected throughout Denmark and screened for rickettsiae by a PanRickettsia-real-time-PCR. Positive pools were analyzed by a real-time-PCR, amplifying all rickettsiae but only identifying *R. helvetica* by a specific probe. Samples yielding PCR-products but negative real-time PCR-results were investigated by Sanger sequencing. A random selection of *Rickettsia*-positive samples was investigated by additional PCR targets for molecular variations within the respective *Rickettsia* species. A total of 367 pools (237n, 66m, 64f) were found positive for rickettsiae, resulting in a minimal infection rate (MIR) of 4.9%. The occurrence of two *Rickettsia* sp. was shown: *R. helvetica* (MIR 4.27%) and, for the first time in Denmark, *R. monacensis* (MIR 0.03%). In the different collection sites, MIR for *R. helvetica* ranged from 0.0%–9.09%; for *R. monacensis* from 0.0%–0.28%, respectively. No variability of the nucleotide sequences between the samples and in comparison, with sequences from rickettsiae sampled in Germany was observed. In summary, minimal infection rates of 4.27% for *R. helvetica* and 0.03% for *R. monacensis*, were found in Denmark. Both *R. monacensis* positive pools were located in the north, indicating the possibility of a limited and focal occurrence. As 13.6% of the positive pools were not differentiable, the occurrence of other, maybe so far unknown rickettsiae should be investigated.
Rickettsia parkeri is an emerging human pathogen transmitted by Amblyomma ticks in predominately tropical and subtropical regions of the western hemisphere. In 2014 and 2015, 1 confirmed and 1 probable case of Rickettsia parkeri rickettsiosis were reported from the Pajarita Wilderness Area, a semi-arid mountainous region in southern Arizona. To examine more closely the potential public health risk of R. parkeri in this region, a study was initiated to investigate the pervasiveness of Amblyomma ticks in mountainous areas of southern Arizona as well as to ascertain the infection prevalence of R. parkeri in these tick populations. During July 2016, a total of 182 adult ticks were collected and evaluated from the Pajarita Wilderness Area in Santa Cruz County and 2 additional sites in Cochise and Santa Cruz counties in southern Arizona. DNA of R. parkeri was detected in 44 (24%) ticks. DNA of “Candidatus Rickettsia andeanae” and Rickettsia rhipicephali was detected in 3 (2%) and 1 (0.5%) of the samples, respectively. These observations corroborate previous collection records and indicate that established populations of A. maculatum exist in multiple foci in southern Arizona. The high incidence of R. parkeri in these tick populations suggests a public health risk as well as the need to increase education of R. parkeri rickettsiosis for those residing, working in, or visiting this area.
Dalyte Mardosaite-Busaitiene – *Rickettsia* species in ectoparasites collected from small rodents in Lithuania

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*Rickettsia* species are obligate intracellular gram-negative bacteria responsible for many human infections, causing public health problems in many countries around the world. *Rickettsiae* are found in association with a wide range of arthropods which feed on very different species of large and small animals. Nevertheless, there are only few studies focusing on investigation of rickettsial pathogens in rodent ectoparasites. This study is the first reports of *Rickettsia* species in ticks, mites and fleas collected from six different species of small rodents in Lithuania. A total of 1263 ectoparasites were collected from 238 small rodents in Curonian Spit (Lithuania) during 2013-2014. Rodents were found to be infested with *Ixodes ricinus* ticks (n=578), 5 species of parasitic mites (n=570) from Laelapidae family (*Laelaps agilis, Hyperlaelaps microti, Haemogammassus nidi, Eulaelaps stabularis, Myonyssus gigas*) and 8 species of fleas (n=115) (*Ctenophthalmus agyrtes, Ct. assimilis, Hystrichopsylla talpae, H. orientalis, Megabothris turbidus, M. walkeri, Palaeopsylla soricis, Nosopsyllus fasciatus*). *Rickettsia* DNA was detected in 73.6% *I. ricinus* ticks, 43.5% fleas and 34.3% *L. agilis* mites. Infected ectoparasites were found on *Apodemus flavicollis, Micromys minutus, Myodes glareolus and Microtus oeconomus* rodents. PCR and sequence analysis of partial *glt*A and 17kDa genes revealed the presence of *R. helvetica*, *R. raoulti* in *I. ricinus* ticks and mites, and *R. helvetica* and rickettsial endosymbionts in fleas. The results of the study demonstrate high infection rate of spotted fever group rickettsiae in ticks and fleas and suggest that ectoparasitic mites may be reservoirs of pathogenic rickettsiae.
Rickettsiae have worldwide occurrence and rickettsiosis are widely recognized as emerging infections in several parts of the world. For decades, it was thought that a single pathogenic tick-borne spotted fever group (SFG) *Rickettsia* occurred on each continent. Moreover, different *Rickettsia* species isolated from ticks were not considered pathogenic for humans throughout years or decades until a definitive association with human disease was established, such as the emblematic case of *R. massiliae* in Europe. In a framework of diagnostic activities of the Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale” and considering some reports of suspected human clinical cases of rickettsiosis, a survey on ticks collected from animals and humans was carried out aimed to identify the *Rickettsia* species circulating in Abruzzo and Molise regions. One hundred and seventy eight tick samples previously identified at developmental stage and species level by morphology (*D. marginatus, I.ricinus, I. hexagonus, R. turanicus, R. sanguineus, H. marginatum* and *Hae.punctata* have been identified) were tested as single ticks or pool by pan-*Rickettsia* Real Time PCR. DNA from specimens turned out to be positive for *Rickettsia* spp. (49%) was then sequenced in order to identify the *Rickettsia* species involved. The selected target for the purpose were *OmpA* and *gltA*. *Rickettsia monacensis, R. massiliae, R. conorii, R. aeschlimanni, R. helvetica, R. raoultii, R. slovaca* and *R. felis* were identified in this study. These are the first data available in literature for the circulation of SFG Rickettsiae species in the selected geographical area. Results made evidence of high rate of infection in ticks by different *Rickettsia* species all involved in human infection as described in literature. Association between *Rickettsia* species, ticks and the relationships with vertebrate host species are discussed. In order to assess the risk for humans and to elucidate more precise etiological diagnosis in clinical cases the use of diagnostic tools able to identify *Rickettsia* at species level is recommended.
Anaplasma phagocytophilum causes an emerging, tick-borne disease of humans in the U.S. but has long been known to infect multiple species globally. The organism is of concern to both veterinary and human medicine and potentially could contaminate blood supplies. Complete or draft genome sequences are now available from 28 U.S. and European strains from different animal species and humans, enabling strain comparisons. It has been suggested that pairwise Average Nucleotide Identity (ANI) between genomes can be used to determine species relationships. Previous analyses of genome databases using Jspecies software led to the proposal that the boundary between species could be set at ~95-96% ANI. Applying this measure to A. phagocytophilum resulted in a maximum ANI of 99.99% and a minimum ANI between strains of 94.41%. Human, dog and rodent strains from the Northeast and Midwestern U.S. were most closely related with ANI >99.71% using either Mummer or Blast comparisons. A supportive method for genome comparisons, which depends only on nucleotide composition without any sequence alignment, is the pairwise correlation of tetranucleotide signatures between genomes (Tetra). The Tetra correlation coefficients between strains varied from 0.9999 to 0.9874, with the same closely related U.S. strains having correlation coefficients >0.999. Taxonomic trees of complete genomes supported these relationships and distinguished particularly two strain groups of either European or U.S. origin. Missing and/or modified genes between groups were identified that could be used to track the global circulation and evolution of different lineages of A. phagocytophilum in their tick vectors and animal hosts.
Experimental infection study in dogs with *Anaplasma phagocytophilum* Korean isolate

Yoon Kyoung Cho, Jun Gu Kang, Young Sun Cho, Jeong Byoung Chae, Sung-Suck Oh, Do-hyeon Yu, Joon Seok Chae

Human granulocytic anaplasmosis (HGA), an emerging infectious disease in the Republic of Korea (ROK), is caused by *Anaplasma phagocytophilum*. Recently, *A. phagocytophilum* has been firstly isolated from human and rodent in Korea. In this study, we performed experimental infections in dogs with *A. phagocytophilum* isolated from rodent. Six beagles were inoculated intravenously with *A. phagocytophilum* propagated in THP-1 cells. Animals were monitored for 16 and 32 days to evaluate infection-specific clinical symptoms, complete blood count (CBC), serum chemistry, serology (using a newly developed prototype from our research group) for detecting *A. phagocytophilum* antibodies and pathological features. Although no clinical signs were noted, the microscopic inflammatory lesions were observed in the lungs, livers, lymph nodes, and proximal tubular epithelium of kidneys. The thrombocytopenia, mild decreased pH, and increased level of (C-reactive protein) CRP were observed in all dogs. Although monocytosis was mainly observed on 14 days and 16 days, the levels of monocytes was returned to baseline levels. The reticulocytosis was observed after 20 days. In diagnostic test for *A. phagocytophilum* antibody, antibodies were detected on 8 days in partial dogs and after 10 days in all dogs. This study is the first experimental infection study using Korean *A. phagocytophilum* strain and represents that the newly developed prototype can detect *A. phagocytophilum* antibody in early stages of infection.
Human granulocytic anaplasmosis (HGA) is caused by obligate intracellular bacterium, Anaplasma phagocytophilum. Features of human infection range mild to severe diseases such as flu like symptoms, acute fever, and renal failure or neurological problems. Human granulocytic anaplasmosis (HGA) is a zoonotic disease caused by obligate intracellular bacterium, A. phagocytophilum, transmitted to humans by infected tick bites such as Ixodes scapularis and I. pacificus. In South Korea, there are growing concern about the possible emergence of HGA since reporting A. phagocytophilum patient in 2014. HGA human infections were increased basis on serologic test using IFA methods. We have isolated A. phagocytophilum from two human origins. We investigated the immune responses against A. phagocytophilum of a South Korean isolate via laboratory mice experiment. The proinflammatory cytokines, IFN-γ is necessary for innate immunity and plays an important role in the induction of severe A. phagocytophilum-infected mice and in host animals, humans. Our data shows IFN-γ, IL-6, IL-10, IL-12p20 and TNF-α productions were slightly increased at day 7 post infection compared to mock-infected controls including splenomegaly. In immunophenotyping for quantification of cell subset with A. phagocytophilum infections, CD8+ and CD4+ T lymphocyte populations in order were significantly expanded in spleens of A. phagocytophilum-infected mice compared with mock-infected mice. These results show that our South Korean isolate seems to be infectious playing a role as a kind of pathogen depending on immune responses and T lymphocytes activations. For further study to analyze various immune responses and more exact pathogenicity, it is needed to compare Korean isolate with more other A. phagocytophilum strains.

Acknowledgements: This work was supported by an awards (4800-4845-300-210-13) from the Korea Centers for Disease Control and Prevention.
In order to improve sample screening capacity and E. ruminantium molecular diagnostics, an automated DNA extraction method for Amblyomma ticks and a new qPCR targeting the pCS20 gene region were successfully developed. A comparison between the new pCS20 Sol1 qPCR, a previously published pCS20 CowTqM qPCR and the pCS20 nested PCR was carried out. pCS20 Sol1TqM qPCR was found to be as specific as the nested PCR but with limited risk of sample contamination and less time-consuming. Further, it did not detect Rickettsia, Anasplasma and Babesia species nor Panola Mountain Ehrlichia, E. chaffeensis and E. canis. The whole method, including the automated DNA extraction and pCS20 Sol1TqM qPCR, demonstrated to be sensitive, specific and highly reproducible with the same limit of detection as the manual DNA extraction and nested PCR. Finally, it allows for 96 samples to be tested in one day compared to four days for manual DNA extraction and nested PCR. The development of a new automated DNA extraction using a DNA/RNA viral extraction kit and qPCR enhances the accuracy of E. ruminantium epidemiological studies performed on tick samples, as well as allowing for the improvement of diagnostic capabilities and enhanced turn-over time for heartwater surveillance. In addition, the new method opens new opportunities for large-scale screening of other bacteria and viruses in ticks as well as tick genetic characterization and co-evolution studies.
Variety of *Chlamydia* species belonging to the *Chlamydiaceae* family were reported in reptilian hosts but no data about their occurrence in Invasive Alien Species (IAS) turtles and tortoises is available. In this study, a research was conducted to acquire information on IAS and other turtle and tortoises, living both in nature and captivity, as possible reservoir of *Chlamydiaceae*. Analysis of specimens (pharyngeal and cloacal swabs, tissues) from 205 turtles revealed an overall *Chlamydiaceae* prevalence of 19.4% and 28.6% among free-living and captive animals, with variable levels of shedding. Further testing conducted with species-specific real-time PCR and microarray test were unsuccessful. Subsequently sequencing was applied to genotype the *Chlamydiaceae*-positive samples. Almost full length of 16S rRNA and *ompA* genes as well as the 16S-23S intergenic spacer (IGS) and 23S rRNA domain I were obtained. Phylogenetic analysis of 16S rRNA amplicons revealed three distinct genotypes. Genotype 1 (ten specimens), specific for freshwater turtles and reported for the first time, was most closely related to *C. pneumoniae* strains and the newly described *Cand. C. sanzinia*. Genotypes 2 and 3 (four specimens), detected in *Testudo* spp. samples, showed highest homology to *C. pecorum* strains but formed a separated sub-branch. Finally, deep molecular analysis of DNA isolates together with isolation from animals living in geographically distant places strongly imply this to be a new species in *Chlamydiaceae* family. In depth studies of *Chlamydia* spp. from turtles and tortoises are needed to further characterise these atypical strains and address arising questions about their pathogenicity and zoonotic potential.
Virulence factors are known to be secreted by intracellular bacteria to interact with their hosts. For classical Chlamydiaceae, several effector proteins were described to be able to modulate various host cell functions, leading to survival and replication of the bacteria. On the other hand, nothing is known about effector proteins secreted by Chlamydia-related bacteria, such as Waddlia chondrophila, an emerging pathogen for humans and animals. We used a lysin to specifically permeabilize host cell membrane to identify effector proteins specifically secreted in the host cell cytoplasm. By mass spectrometry analysis, we could identify 31 bacterial proteins present in the host cell cytoplasm and released in the supernatant after permeabilization. We did not elucidate the mode of secretion of all of them but we could identify a Type III Secretion System (T3SS)-dependent secretion for at least two of these bacterial proteins.

The second approach was to identify, in silico, effector proteins secreted by T3SS. Four algorithms were used to predict a few proteins as secreted by T3SS with a good probability. Secretion was confirmed using an heterologous system. Chaperone proteins are required by T3SS effectors for correct folding before secretion within host cell, and these chaperones exhibit high sequence homology. The last approach was to use these chaperones to bind effectors in a pull down assay analysed by mass spectrometry. We then increased the number of putative virulence identified.

This large screening led to the identification of new chlamydial secreted effector proteins, on which we are carrying out further investigations to increase our understanding of chlamydial evolution, biology and pathogenesis.
Inflammasomes are key multiproteic complexes assembled in response to several stimuli such as pathogenic microorganisms. Once activated, inflammasomes induce a cascade of signalisation leading to the secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18, and a rapid pro-inflammatory cell death named pyroptosis. C. trachomatis and C. pneumoniae are known to induce NLRP3/ASC inflammasome, but virtually nothing is known about relationship between Chlamydia-related bacteria and this signalling pathway. Parachlamydia acanthamoebae, Waddlia chondrophila, and Estrella lausannensis have the ability to enter and grow more or less efficiently within macrophages. In our model of mouse bone marrow-derived macrophages (BMDM), the 3 bacteria are able to enter cells, but only W. chondrophila is able to replicate efficiently. Despite its inability to grow within these cells, P. acanthamoebae is able to induce cell mortality by apoptosis, in less than 36 hours. E. lausannensis induces a dramatic cell death up 80 % in only 4 hours. This cell death is associated to cleavage of Caspase-1, IL1-β, and IL18, and secretion of IL1-β, and IL18. Cell death present pyroptotic features. We are still trying to identify which inflammasome is involved, but it is not the same than those activated by classical chlamydiae. Further investigations are needed to understand the differences between bacteria of the Chlamydiales order leading to inflammasome activation or inhibition, and then to understand their pathogenicity towards humans and animals.
P1-8) Trestan Pillonel – Taxogenomics of uncultured chlamydiae

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The phylum Chlamydiae forms a deep branching clade within bacteria. All known members of the phylum are obligate endosymbionts of eukaryotes. Metagenomic studies of environmental samples suggest a high diversity of chlamydial species, but only few representative strains were extensively described because of the difficulty to culture them. Genome sequencing provides an alternative approaches to investigate the diversity, biology and evolution of the phylum. An increasing number of unclassified genomes exhibiting similarity to sequenced chlamydial genomes are submitted to public databases. The aim of this project is to perform whole genome comparative analysis of uncultured and cultured chlamydial strains in order to refine the taxonomy of the phylum and to investigate its metabolic diversity. Thirty-six genomes from 7 family-level lineages and 21 metagenomes were included in the analysis. Proteins were clustered into orthologous groups using Orthofinder. The genome quality was assessed using nearly universal proteins. Metabolic capacities were evaluated using the KEGG database, and proteins domains were identified using Interproscan. Metagenomics bins exhibited uneven quality, but only 3 assemblies estimated to represent less than 90% of the complete genome. Phylogenetic reconstruction based on single copy orthology suggests the existence of 8 new clades, including 4 new family level lineages. Comparative analysis of predicted metabolic capacities indicated that chlamydial family level lineages do not reflect coherent groups with distinct metabolic profiles. With the identification of at least 4 yet undescribed family level lineages of the phylum Chlamydiae, this work highlights the promises of environmental metagenomics for the study of uncultured obligate symbionts of eukaryotes.
The aim of the study was to analyze the phenotypic and genotypic resistance of C. trachomatis isolates to some antibiotics. For the test, 57 patients with a diagnosis of chlamydia-induced arthropathy were selected. All patients had previously undergone antichlamydia therapy without a positive microbiological and clinical effect. Phenotypic resistance was determined on the culture of McCoy cells using average therapeutic concentrations of antibiotics. Genotypic resistance in C. trachomatis consisted in the detection of tetra- and erm-gene fragments using PCR analysis.

In all patients the chlamydia infection was laboratory confirmed. However, 11 C. trachomatis isolates were not maintained in subpassages on McCoy cells, due to the active growth of opportunistic microorganisms and herpesviruses. Thus, only 46 isolates of C. trachomatis were analyzed.

When evaluating phenotypic resistance, the most frequently detected resistance to doxycycline - 22 (47.8%) of 46 isolates, tetracycline - 14 (30.4%), and ofloxacin - 13 (28.3%) isolates. Resistance to several antibiotics simultaneously showed in 22 (47.8%) isolates.

In a parallel study of genotypic resistance to tetracyclines and macrolides, a tet-gene was detected in 36 (78.3%) C. trachomatis isolates, and erm-gene was detected in 19 (41.3%). The tet-gene and erm-gene were simultaneously detected in 13 (28.3%) isolates.

Comparative analysis showed that in 32 (69.6%) of 46 cases, correlations between phenotypic and genotypic resistance to antibiotics among the isolates were found.

Thus, identified C. trachomatis isolates resistant to the various antichlamydial drugs (doxycycline, tetracycline, azithromycin, ofloxacin etc.).
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In the past decades, various Chlamydia-related bacteria have been discovered, including Simkania negevensis that was discovered in Israel in 1992 as a cell culture contaminant. Previous reports have reported a high seroprevalence of S. negevensis worldwide and an association with respiratory diseases, such as pneumonia and bronchiolitis, has been suggested. On the other hand, recent studies have failed to show a significant association, leaving doubt about the clinical relevance of this emerging bacterial pathogen. The lack of specific diagnostic tools has hammered out definite conclusions. Thus, we developed a specific quantitative real-time TaqMan polymerase chain reaction (PCR), which was subsequently applied to 477 clinical samples (respiratory, urogenital, cardiac and hepatic samples) taken from patients hospitalized in Lausanne (Switzerland). In parallel, a seroprevalence survey, based on a micro-immunofluorescence assay, was performed among 683 European pregnant women and 105 asymptomatic Swiss young men; recommended positivity cut-offs were used (titer ≥1:64 for IgG; ≥1:32 for IgM).

All the 477 samples tested negative for S. negevensis by PCR. These results were confirmed by a pan-Chlamydiales PCR used as gold standard. Congruently, a 0.5% (4/788) seroprevalence was observed suggesting a low exposition to S. negevensis among the tested population. This contrasted with previous reports. Though geographical characteristics and specific exposure in some populations may explain these variations, the higher specificity of the diagnostic tools and cut-offs used could alternatively explain these discrepancies. We conclude that S. negevensis is not clinically relevant in Western Europe.
Chlamydia gallinacea is an obligate intracellular bacterium found in commercial poultry farms. It was added as a new species to the genus Chlamydia in 2014 and its avian pathogenicity or possible zoonotic potential has yet to be elucidated. In 2015 and 2016, as part of a surveillance for zoonotic pathogens in farm animals in the Netherlands, pooled faecal samples from 151 randomly selected layer farms were investigated for the presence of Chlamydia DNA. C. gallinacea was detected on 73 farms and a subset of the positive samples was selected for ompA gene sequencing to gain further insight in existing genetic diversity. Briefly, primers were designed that covered all four variable domains (VDs) of the ompA gene. The amplicon was purified and subsequently sequenced. The resulting sequences were analysed and finally the amino acid sequences of variable domain 2 and 3 were aligned and compared with published C. gallinacea ompA sequences. None of the sequenced variable domain 2 and 3 were identical to the published sequences and at least 7 new genetic variants could be identified. These findings are in line with other publications showing the ompA gene of different C. gallinacea isolates is highly polymorphic. It suggests C. gallinacea is not clonally spread over layer farms in the Netherlands and might have been present over a longer period of time. Further work will be performed to identify any within-farm or even within-animal variety in the C. gallinacea ompA gene in relation to pathogenicity and possible zoonotic potential.
Bartonella genus contains more than 30 different species of gram-negative intracellular bacteria that infect a wide variety of mammalian hosts. Many different species of hematophagous arthropods, including deer keds, have been confirmed as important vectors in Bartonella transmission. This study represents the first record of deer keds from cervids with presence of Bartonella pathogens in Lithuania. Taxonomic identification of deer keds was based on description of morphological characteristics of pupae and adults and sequence analysis of mitochondrial COI gene. Our investigation demonstrates that in Lithuania cervids are infested with two different species of deer keds Lipoptena cervi and L. fortisetosa. The highest infestation rate was detected in moose (Alces alces). Nested PCR was used to amplify 16S-23S rRNA intergenic spacer region (ITS) of Bartonella genus. Bartonella DNA was detected in both species of deer keds with high prevalence. Sequence analysis of ITS region showed close similarity of detected pathogens to Bartonella schoenbuchensis.
Bartonella quintana, a pathogen that is restricted to human hosts and body louse vectors, was characterized for the first time as the agent of trench fever. This bacterium is now recognized as a reemerging pathogen, mostly among homeless populations in diverse regions of the world. Body louse (often prevalent among homeless) is considered to be a vector, although recently several manuscripts reported the presence of B. quintana in head lice also. A range of other clinical manifestations are also attributed to B. quintana infection, such as angiomatosis, endocarditis, chronic bacteremia and lymphadenopathy. In Algeria, few studies have been realized previously for the investigation of body louse-borne pathogens and no evidence of the presence of B. quintana is reported. In this study, using molecular techniques, real-time polymerase chain reaction (qPCR) combined with conventional PCR and sequencing we investigate the presence of B. quintana in body lice collected on homeless people in three different localities from Algeria. Results show that the screening of body lice samples reveal the identification of the dangerous human pathogen, B. quintana, in 50 (9.72%) of the 514 body lice collected on 20 (39.21%) of the 51 infested homeless from the three different localities in Algeria. This study reports for the first time the molecular detection of B. quintana in body lice collected on homeless people from Algeria, North Africa. Our results should strongly encourage epidemiological investigations and survey of louse-associated infections to establish the prevalence of louse-borne infections and to better understand the role of body lice in transmission of this agent in humans from Algeria.
P1-14) Mustapha Dahmani – The whole genome analysis-based description of Bartonella mastomydis isolated from Mastomys erythroleucus in Senegal

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The facultative intracellular bacteria Bartonella spp. are small, vector-transmitted Gram-negative homoeotropic bacilli classified within the class Alphaproteobacteria. The genus was significantly expanded after Brenner et al. proposed to unify Bartonella and Rochalimaea genera in 1993, and the unification of Bartonella and Grahamella genera by Birtles et al in 1995. These bacterial species are transmitted between the reservoir and the final mammal host by hematophagous arthropods and insects usually by their bites. Parasitism by Bartonella is widespread among small mammals, and potentially new Bartonella species from commensal rodents were reported from many countries in the world. In February 2013, rodents and insectivores were captured alive in two sites Dielmo and Ndiop in the Northern region of Senegal using wire mesh traps baited with peanut butter or onions. The aim of this study was to investigate the presence of Bartonella spp. in commensal rodents in Sine-Saloum region of Senegal where rodent-associated soft ticks are vectors of relapsing fever caused by Borrelia crocidurae. Trapped rodents and insectivores were anesthetized and opened in sterile conditions. Blood was inoculated on Columbia agar plates (5% sheep blood). In total, during the period of 6 days 119 small mammals were captured: 116 rodents and 3 shrews (Crocidura cf. olivieri). Rodents were identified morphologically: 5 Arvicanthis niloticus, 56 Gerbilliscus gambianus, 49 Mastomys erythroleucus, 5 Mus musculus and 1 Praomys daltoni. 30 isolates of Bartonella spp. were recovered from the blood of rodents. From blood sample of Mastomys erythroleucus, 21 isolates of a Bartonella species were recovered, these isolates are almost genetically identical. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry protein analysis and subsequent genome sequencing and whole genome mapping efforts coupled with results from multilocus sequence typing of the strain 008 using the gltA, rpoB, 16S rRNA, ftsZ genes and the intergenic spacer ITS confirmed the isolates to be unique and corresponded to criteria for a new species proposed by La Scola (2003). Additionally, phenotypic, microscopic, colonial morphology and growth characteristics were observed among the three isolates and, these characteristics were consistent with those found among members of the genus Bartonella. Gram staining and transmission electron microscopy showed the isolates have a mean length and width of 1369.8±423.8 nm and 530.9±105.8 nm. Genomic DNA of the strain 008 was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with two applications, paired end and mate pair. The genome is 2 044 960 bp long with 38.44 % GC content. Of the 1 716 predicted genes, 1 674 were protein-coding genes and 42 were RNAs. Based on these results, all 21 isolates including type strain 008 are considered to be a unique Bartonella agent, provisionally named here Bartonella mastomydis.
Hepatosplenic Cat Scratch Disease (HS-CSD) in immunocompetent adults is a rare condition that has been recently reviewed. Here, we report our experience in diagnosis and therapeutic management in 8 cases (3 previously reported) of this syndrome and compare with 39 cases reported to date in literature. Our patients were older (mean age 60 vs 44 years) and with female predominance (75% vs 45%); peripheral adenopathy were detected more frequently at presentation (50% vs 26%) and presence of abdominal adenopathy in the imaging studies (75% vs 28%). No patient had associated endocarditis or osteomyelitis. The mean duration of antibiotic treatment in our patients was shorter (2 vs 4 weeks); and this data was not modified by the exclusion of patients with endocarditis or osteomyelitis in analysis. Antibiotic monotherapy (62% vs 41%) and a 5 days course of azithromycin (50% vs 3%) were used more frequently in our patients. The clinical resolution was achieved more quickly (3 vs 5 weeks) but the cure (radiologic resolution of lesions) was slower in our patients (5 vs 3 month). The subgroup of patients treated with a 5 days course of azithromycin compared with other antibiotics regimens globally (in monotherapy or in combinations) had more rapid clinical resolution. Based in our experience, we think that a short course of azithromycin is a good choice for immunocompetent patients with HS-CSD not associated with endocarditis, osteomyelitis or nervous central system involvement.
P1-16) Nadia Amanzougaghene – Detection of several emerging bacterial pathogens in human dead lice from Mali

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In poor African countries, where no medical and biological facilities are available, the identification of potential emerging pathogens of concern at an early stage is challenging. Head lice, Pediculus humanus capitis, have a short life, feed only on human blood and do not transmit pathogens to its progeny. It is therefore a perfect tool for xenodiagnosis of current or recent human infection. The present work contributes to this promising approach, by a study of bacterial pathogens associated with head lice collected in two rural villages from Mali, where high frequency of head lice infestation was previously reported, using molecular methods. Results show that all the 600 head lice, collected from 117 individuals, belonged to clade E, specific to West Africa. Bartonella quintana, the causative agent of trench fever, was identified in 3 of 600 (0.5%) head lice studied. Our study also shows, for the first time, the presence of the DNAs of two pathogenic bacteria namely: Coxiella burnetii (5.1%) and Rickettsia aeschlimannii (0.6%) being detected in human head lice, as well as the DNAs of potentials new species from Anaplasma and Ehrlichia genus of unknown pathogenicity. The finding of several Malian head lice infected with B. quintana, C. burnetii, R. aeschlimannii, Anaplasma and Ehrlichia is alarming and highlight the need of an active survey programs to define the public health consequences of these emerging bacterial pathogens detected in human head louse.

Keywords: Pediculus h. capitis, Clade E, Bartonella quintana, Coxiella burnetii, Rickettsia aeschlimannii, Anaplasma, Ehrlichia
The aim of this study was to perform molecular detection and characterization of *Bartonella* spp. and *Rickettsia* spp. in fleas collected from cats in Southern Chile. Fleas (n=150) were obtained from 150 cats in Valdivia city, identified using taxonomic keys and individually submitted to conventional PCR (cPCR) for *Ctenocephalides felis* 18SrRNA gene. cPCR assays targeting *Bartonella* spp. *gltA* and *Rickettsia* spp. *ompA* genes were performed. Selected positive samples of *Bartonella* (n=19) and *Rickettsia* (n=14) were sequenced for species differentiation, phylogenetic and diversity analyses. All fleas were morphologically identified as *C. felis* and positive in 18SrRNA gene cPCR. *Bartonella* and *Rickettsia* occurrences in *C. felis* fleas were 39.3% (59/150) and 76.6% (115/150), respectively. From sequenced *Bartonella* spp., 42.1% (8/19) were identified as *Bartonella henselae*, 47.3% (9/19) as *B. clarridgeiae*, 5.3% (1/19) as *B. koehlerae*, and 5.3% (1/19) as *Bartonella* sp., presenting 99% identicalness to a raccoon *Bartonella* sp. *Rickettsia felis* was the only *Rickettsiaceae* species (14/14) identified. Five haplotypes of *B. henselae*, closely positioned to other *B. henselae* from Chile, Brazil, Houston and New Caledonia, were detected with 4 variable sites and a nucleotide diversity of $P_i=0.001$; *B. clarridgeiae* presented 5 haplotypes, 8 variable sites and $P_i=0.003$. *Bartonella koehlerae* was closely positioned with a sequence from Chile. *Rickettsia felis* presented 7 haplotypes, 7 variable sites and $P_i=0.002$. *Bartonella* spp. and *R. felis* are described for the first time in *C. felis* fleas from Southern Chile, with moderate to high frequencies, highlighting the importance of vectors as a source of zoonotic agents.
Lymph node enlargement is a common medical problem that is usually caused by bacterial, viral, fungal, and protozoal agents. The clinical symptoms are often similar or nonspecific, and several etiological agents could be responsible for enlarged lymph nodes. *Bartonella henselae*, the agent of cat scratch disease (CSD), appears to be the most common organism responsible for lymphadenopathy, both in adults and children. We analyzed 2,170 lymph node biopsy samples from patients with suspected infectious lymphadenitis from January 2013 to December 2016 by qPCR for *B. henselae*, *Coxiella burnetii*, *Francisella tularensis*, *Mycobacterium* sp. and *Tropheryma whippelii*. In addition samples were tested by PCR and sequencing of the 16S ribosomal DNA gene and by culture. Among the 673 positive samples, *B. henselae* was the most common bacterial agent (n= 441, 66%), followed by *F. tularensis* (n=45, 7%) and *Mycobacterium* sp. (n=32, 5%). *Mycobacterium tuberculosis* and *Mycobacterium avium* were the most mycobacteria detected (n=22, 3% and n=5, 0.7% respectively). Seven (1%) lymph nodes were positives for *T. whippelii*. The amplification of the 16S rDNA gene was positive for 286 patients (43%), and *B. henselae* was the most frequently amplified bacterium (129 cases, 19%) followed by *Staphylococcus aureus* (43 cases, 6%). Overall 42 different bacteria were identified by 16S rDNA. Our study demonstrates the usefulness of qPCR for the molecular analysis of lymph node samples but as we cannot exclude infections by other new or emerging bacterial agents the use of 16S rDNA PCR remains critical and should be used in the strategy of lymph node examination.
P1-19) Laura Franzin – Preliminary results of intracellular bacteria detection from culture-negative endocarditis patients in North-West Italy

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Coxiella and Bartonella spp. are most frequent intracellular bacteria from blood-culture negative infective endocarditis (IE). Aim of the study is to investigate Bartonella, Coxiella and other intracellular bacteria in human clinical samples of culture-negative endocarditis patients in Piedmont Region, North-West Italy. 152 samples (blood EDTA, plasma, buffy coat, serum) from 48 patients with suspected IE were examined. Bartonella DNA was amplified by PCR (ITS). Blood samples for Bartonella culture were inoculated onto Columbia and BHI blood agar plates and in BBH liquid medium, followed by subcultures on blood agar plates, Legionella culture was performed on BCYE agar plates. PCR was also performed for Coxiella (Trans-PCR IS1111), Legionella (real-time PCR 5SrRNA), Tropheryma whipplei (16SrRNA), Listeria monocytogenes (real-time PCR hly) and Rickettsia (gltA). Antibodies against C. burnetii, B. quintana and B. henselae were determined by IFA. Bartonella DNA was detected from two patients with endocarditis (one subject was homeless with positive serology). All cultures were negative for Bartonella and Legionella. Coxiella DNA was positive in one patient. Legionella, Listeria, Rickettsia and Tropheryma PCR were negative from blood of all patients. However, Tropheryma whipplei DNA was positive (confirmed by sequencing) in feces and in duodenal biopsy of a patient with endocarditis and suspected Whipple’s disease. Little is known about Bartonella, Coxiella and Tropheryma whipplei endocarditis in Italy and the incidence may be underestimated in both immunocompetent and immunocompromised patients. Despite the limited number of patients examined, these pathogens were detected in Piedmont Region suggesting that greater attention should be given to these intracellular bacteria.
DEBONEL (Dermacentor borne necrosis erythema lymphadenopathy)/TIBOLA (tick-borne lymphadenopathy), is a tick-borne rickettsial disease transmitted by Dermacentor spp. described in Europe during the past two decades. This syndrome is also called SENLAT when the tick-bite is on the scalp. Dermacentor marginatus is the main vector, although Dermacentor reticulatus has been also involved. Rickettsia slovaca, Candidatus Rickettsia rioja and Rickettsia raoultii have been implicated as etiologic agents. The aim of this study was to determine Rickettsia spp. present in D. marginatus removed from DEBONEL/TIBOLA patients. From January 2001 to December 2015, a total of 70 D. marginatus of 157 patients were analyzed. The presence of SFG Rickettsia was determined by PCR assays for ompA, ompB and gltA genes. All Dermacentor ticks analyzed were infected with Spotted Fever Group (SFG) Rickettsia. In 40 D. marginatus we amplified Ca. R. rioja (57.1%); in 28, R. slovaca (40%); and R. raoultii was only amplified in two D. marginatus ticks (2.8%). In addition, sequences homologous to Rickettsia sp. DmS1 (100% identity) were detected after analysis of gltA amplicons corresponding to 10 D. marginatus that harboured Ca. R. rioja (according to ompA and ompB genes).

Regarding sensitivity, ompA gene PCR allowed us to detect Rickettsia sp. in 91% of ticks, ompB gene PCR, in 87.5% and gltA gene PCR, in 66.6%. Ca. R. rioja, R. slovaca and R. raoultii were the SFG Rickettsia found in DEBONEL/TIBOLA ticks. An uncultured Rickettsia sp. DmS1 was detected using gltA target gene in D. marginatus from DEBONEL/TIBOLA patients.
Spotted fever group (SFG) and typhus group (TG) rickettsial infections are distributed worldwide. However, these infections are not suspected in patients with undifferentiated fever or reported nationally in Malaysia. To ascertain the frequency of rickettsioses, we prospectively enrolled patients in Kudat and Kota Marudu district hospitals in northern Sabah, East Malaysia (Borneo) from Dec 2013-Jan 2015. Patients febrile within 48h of admission and with negative malaria blood smears were eligible. Acute and convalescent sera and whole blood (wb) or buffy coat (bc) were obtained. Convalescent sera were screened by IFA for SFG (Rickettsia conorii) and TG (R. typhi) IgG. Acute phase mates of IgG-reactive convalescent samples were also tested. Acute phase IgM IFA was done when convalescent samples were unavailable and on 120 sera with paired IgG results. A 4-fold IgG titer rise to ≥160 confirmed acute infection and a <4 fold IgG titer rise past infection; those IgM-positive alone were probable. Multiplex qPCR (mqPCR) targeting SFG and TG rickettsiae was conducted using DNA from wb or bc. Of 121 and 122 paired samples tested for SFG and TG, respectively, 4-fold increases in IgG titer were observed in 8 (6.6%) and 4 (3.3%); 6 (5.0%) and 8 (6.6%), respectively, had <4 fold IgG titer changes. Of 148 acute sera without paired IgG testing, 3 (2.0%) and 5 (3.4%) were IgM-positive, respectively. Among samples with paired IgG, IgM identified 1 additional possible SFG rickettsiosis. Overall, 26/115 (22.6%) sera had evidence of confirmed acute (12/115 [10.4%]) or past (14/115 [12.2%]) SFG or TG rickettsioses in a small region of East Malaysia. Of 110 wb and 147 bc samples tested by mqPCR, SFG and TG rickettsioses were detected in 3 (1.2%) and 2 (0.8%), respectively. One TG mqPCR+ sample had acute phase IgM and IgG titers of ≥2560 but no convalescent sera. As clinical suspicion guides management in these regions and rickettsioses require specific treatment, a comprehensive prospective study of SFG and TG in Malaysia is warranted to help define risk across Southeast Asia.
**P1-22) Lukas Frans Ocias – Rickettsiosis in Danish patients suspected of Lyme neuroborreliosis**

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**Background:**
*R. helvetica* can be found in 4.7-13\% of Danish *Ixodes ricinus* ticks making it the second most frequent pathogen found in ticks in Denmark (1,2). Most reports of human infection have reported a mild, self-limiting flu-like illness but it has also been associated with subacute lymphocytic meningitis (3,4). As both *Borrelia* and *R. helvetica* are found in the same tick species, potential co-transmission is a possibility. We have examined the seroprevalence of antibodies against *Rickettsia* in 192 patients suspected of Lyme neuroborreliosis. Furthermore, we have examined the cerebrospinal fluid (CSF) of same patients for the presence of *Rickettsia* using PCR.

**Methods:** A total of 192 samples consisting of sera and CSF were used for this study. All samples had prior to our study been tested for neuroborreliosis using the intrathecal anti-*Borrelia* antibody index (AI) test, providing us with 98 AI positive and 94 AI negative patients. Sera from these patients were tested using a commercially available indirect immunofluorescence assay (IFA) from Focus diagnostics (IF0100G, Rickettsia IFA IgG; Focus Diagnostics, Inc., Cypress, CA, USA) which uses antigens from *R. typhi* and *R. rickettsii* to discriminate on the genus-level between SFG and TG rickettsia. Titers at or above 1:64 were considered positive for IgM and for IgG a titer at or above 1:512 was indicative of a positive result. Corresponding CSF from 189 patients (95 AI positive and 94 AI negative patients) was further tested for rickettsia using a genus-level real-time PCR previously described by Stenos et al. (5) and if, positive, followed by nested PCR for the 16 sRNA, ompB, gltA and 17-kDa genes. Positive PCR samples were sent to sequencing. All samples tested were archival specimens stored in a biobank at Statens Serum Institut in Copenhagen, Denmark, in -20\(^\circ\) freezers and collected during the months of July to October in the years 2010-2015. As negative controls, we used 106 plasma samples from healthy blood donors (6) and 100 samples of CSF without pleocytosis, collected from the department of clinical microbiology at Slagelse Hospital.

**Results:**
18 of the 192 patients (9\%) had detectable antibodies against SFG rickettsia. The prevalence was higher in the AI positive group (12/98 = 12\%) compared to the AI negative group (6/94 = 6\%), but this was statistically insignificant (p = 0.25). 11 of the patients tested positive for both TG and SFG rickettsia but none had a higher titer for TG than SFG rickettsia. We are still awaiting results for the conventional PCR and sequencing of the CSF samples.
Conclusion: No significant difference in the seroprevalence of antibodies against SFG rickettsia was seen between the AI negative and the AI positive patients. The 12% seroprevalence among the AI positive patients could be indicative of a higher degree of exposure and its significance needs to be further investigated.

Acknowledgements:
We would like to thank the diligent laboratory staff at the department of Virus and Microbiological Special Diagnostics at Statens Serum Institute for their kind help with IFA.

References
2. Kantsø B et al. Seasonal and habitat variation in the prevalence of Rickettsia helvetica in Ixodes ricinus ticks from Denmark.
Scrub typhus, is an acute febrile illness caused by the intracellular bacterium Orientia tsutsugamuchi, which is transmitted to humans through the bite of infected Leptotrombidium mites. It is estimated that about one million cases of scrub typhus are reported each year in Asian-Pacific endemic region.

We report a travel-acquired scrub typhus case imported to Portugal, where other rickettsial infections are endemic.

A 57-year-old Portuguese woman, was admitted in Hospital in Lisbon on May 2016. The patient reported a travel history to Macao and Hong-Kong returning 10 days previously to hospital admission. While she was in Lisbon she presented with a 5-day course of fever, with chills, malaise and intense myalgias. At fourth day, she notice the presence of a maculo-papular-nodular rash with palmo-plantar involvement. Physical examination showed additionally a lesion that was compatible with an inoculation site in the right hemithorax.

Analytical evaluation showed an elevation of C-reactive protein, trombocytopenia and mild hepatic cytolysis. Patient started empirical treatment with doxycycline.

After negative results of serological and molecular detection of Rickettsia DNA in patient’s eschar biopsy and since the patient was traveling in Asia, it was performed the diagnosis for Orientia. Using PCR/sequencing and primers targeting the gene that encodes the 56-kD protein it was confirmed the diagnosis of the infection by Orientia tsutsugamushi.

In spite of the clinical picture that can be attributed to a different rickettsial infections and the empirical treatment common to both, the described case illustrates the relevance of the epidemiological history and laboratory diagnosis to identify the etiological agent.
To explore microbial diversity in ticks with affinity to bite humans can contribute to a more accurate diagnosis of tick-borne diseases (TBD). Our aim was to characterize the bacterial microbiome of tick species that bite humans in La Rioja (North of Spain). Thirty adult ticks (*Ixodes ricinus*, *Dermacentor marginatus*, *Rhipicephalus sanguineus* and *Haemaphysalis punctata*) from vegetation in La Rioja were studied. Pair-end sequencing of the hypervariable V3 and V4 regions of the 16S rRNA gene was conducted using the Illumina MiSeq platform. The V3-V4 region (550-580 bp) was reconstructed through paired reads using QIIME. Four sample groups were defined for analysis, according to the four tick species. The Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Proteobacteria, Tenericutes and Verruromicrobia phyla were observed in all groups. Proteobacteria was the dominant phylum in all species. Within Rickettsiales (4.8% abundance), *Rickettsia* (1.4%), *Wolbachia* (0.5%), *Ehrlichia* (0.5%), *Anaplasma* (0.3%) and other genera (2.1%) were found. *I. ricinus* was the most diverse species (alpha-diversity measurements), followed by *D. marginatus*, *H. punctata* and *R. sanguineus*. Differences between *R. sanguineus* and *D. marginatus* were statistically significant (*P* < 0.01). A clear separation of ticks by species was not observed. Looking for significant differences between OTU abundances, twelve OTUs were found (*P* < 0.01). Ten of them were classified as new references belonging to Rickettsiales, Legionellales or Pseudomonadales (Alpha- or Gammaproteobacteria). High-throughput sequencing seems useful to discover the spectrum of bacteria carried by ticks. More studies are needed to identify and differentiate bacterial species and to improve the knowledge of TBD in Spain.

Acknowledgments: This study was partly supported by a grant from Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Spain (PI15/02269).
This study confirms for the first time the presence of *Rickettsia Slovaca* in wild boar’s (sus scrofa) spleen and their involvement in the epidemiology of rickettsial diseases in Algeria. Indeed, between April 2011 and April 2015, 117 pieces of spleen were removed by laparotomy on wild boars beaten by the approved hunting associations. Total genomic DNA was isolated by using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) and BioRobot EZ1 (QIAGEN) as described by the manufacturer. DNA was used as template for quantitative real-time PCR. We used the RKND03 system, which is specific for the gltA gene of *Rickettsia* spp. Real-time PCRs were performed by using the CFX96 Real Time System C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Singapore). Positive results were confirmed by using a standard PCR specific for the ompA gene of *Rickettsia* spp. We used bacteria-free DNA of *Rhipicephalus sanguineus* ticks reared in our laboratory colonies as a negative control and DNA of *Rickettsia slovaca*, which are known to be associated with *Dermacentor marginatus* ticks, as a positive control. PCR amplification was verified by electrophoresis of products on 2% agarose gels. Products were purified by using a NucleoFast 96 PCR plate (Macherey-Nagel EURL, Hoerdt, France) as recommended by the manufacturer. Purified PCR products were sequenced by using the same primers as for a standard PCR and the BigDye version 1–1 Cycle Ready Reaction Sequencing Mixture (Applied Biosystems, Foster City, CA, USA) in the ABI 31000 automated sequencer (Applied Biosystems). Sequences were assembled and analyzed by using ChromasPro version 1.34 software (Technelysium Pty. Ltd., Tewantin, Queensland, Australia).

We screened ninety-two spleen collected directly on the above mentioned wild boars. Overall, 5.43% (5/92) among them were positive for *Rickettsia* spp. Using a standard PCR specific for the ompA gene of *Rickettsia* spp., we identified *Rickettsia slovaca* (100% similarity 760/760 bp) (GenBank accession no. HM 161787.1).

**Keywords**: *Rickettsia Slovaca*, spleen, Wild boar, Algeria,
Rickettsia species are arthropod-borne obligate intracellular bacteria that cause diseases that impact on medical and veterinary health worldwide. The aim of this study was: a) to identify the presence of Rickettsia species in ticks collected from several sites of Sardinia during 2015; b) characterize the rickettsiae based on sequence analysis of the partial gltA gene. The presence of rickettsial strains was detected in 8.3% of the total adult ticks analyzed (n=156). In detail, this study provides the first evidence of the presence of Rickettsia raoultii, the causative agent of tick-borne lymphadenopathy/Dermacentor-borne necrosis erythema and lymphadenopathy, in 2 D. marginatus and 2 R. sanguineus ticks collected from a wildboar and dogs respectively, in two collection area of the north-western Sardinia. In addition, Rickettsia massiliae was detected in 3 Rhipicephalus sanguineus ticks; Rickettsia slovaca in 2 Dermacentor marginatus, Rickettsia aeschlimannii in 5 Hyalomma marginatum marginatum, 1 Hyalomma lusitanicum and 1 Hyalomma spp. ticks. Detection of R. raoultii in R. sanguineus ticks extends the number of tick species available for the study of the zoonotic pathogen. Moreover, presence of Rickettsia species in ticks collected from birds highlights the importance of wild vertebrates as hosts and carrier of ticks for long distances and contributes to the spreading of tick-borne pathogens. Data increase the knowledge of tick-borne diseases in Sardinia and provide a useful contribution toward understanding their epidemiology.
Skin biopsies from patients with granuloma annulare and morphea, both conditions with unknown etiology, were examined for the presence of Chlamydia-like organisms (CLOs). For both conditions, one of the hypothetic causative agents is *Borrelia burgdorferi* sensu lato, a spirochete that could be transmitted to humans via tick bites. Although the causal connection between *B. burgdorferi* and Granuloma annulare and morphea have been explored in several studies by various methods, including both indirect serological methods as well as direct detection by PCR, the results have remained controversial with no firm evidence of causative role (Zollinger et al 2009, Ranki et al 1994, Valančienė et al 2010). In addition to *B. burgdorferi*, ticks are known to serve as vectors for a number of other animal and human pathogens, such as *Babesia microti* causing babesiosis, *Anaplasma phagocytophilum* causing human granulocytic anaplasmosis, species of the spotted fever group of *Rickettsiae*, and tick-borne encephalitis virus (TBE). In Europe, the most prevalent tick species is *Ixodes ricinus*, and in our earlier study, we detected DNA of Chlamydia-like organisms (CLOs) in approximately up to 40% of adult *Ixodes ricinus* ticks collected in southwestern Finland. In this study, as an attempt to unravel the causative agent of these skin conditions, we examined the prevalence and type(s) of CLO in skin biopsies taken from granuloma annulare and morphea lesions, and compared them to those detected in healthy skin and in ticks collected in Finland (our previously published data). Our results suggest that CLO is prevalent in healthy human skin, but the CLO prevalence is even higher in lesions of granuloma annulare and morphea. Sequence analysis revealed that the distribution of various CLO types differed between the patient groups: the variety of CLO types was bigger in samples from granuloma annulare than in samples from healthy skin or morphea, and the variety of CLOs in the latter two groups was very similar. Moreover, the CLO types detected in granuloma annulare were also detected in *Ixodes ricinus* ticks collected in Finland, suggesting that CLO could indeed be transmitted to humans via tick bite, and may sometimes cause skin conditions like granuloma annulare.
Introduction: Murine typhus (MT) is an acute ubiquitous zoonosis caused by Rickettsia Typhi. It is a flea-borne rickettsiosis, the rat is the most frequent host and his flea the vector. Rickettsia Typhi has been found on indigenous rats. The first case of TM was identified on the island in 2012 and indigenous transmission was proved. At this time, a prospective study was started with the intention of describing clinical, biological and epidemiological manifestations of TM diagnosed since 2012.

Materials and methods: This is a prospective, observational cohort study of MT cases in the Reunion Island from early 2012 to February 2017. The cases were defined with specific PCR and/or serologic positive. Only confirmed cases were included. The clinical, biological and epidemiological data were collected in a standardized questionnaire completed by practitioners in charge of cases.

Results: Sixty one patients were included. Sex ratio was 1.54 (37 men and 24 women). The average age was 42.0 years. The hospitalization rate was 79% (n=48). The main clinical features were prolonged fever ≥ 7 days (90%, n=55), asthenia (87%, n=53), headaches (79%, n=48), arthromyalgia (77%, n=47), pharyngitis (39%, n=24), a maculo papular rash (38%, n=23). The classic triad (fever-headache-rash) was present only in 31% of cases (n=19). Digestive manifestations were present in 48% of cases (n=29). Respiratory manifestations were shown in 30% of cases (n=18), mainly non-productive caught and pneumopathy; neurologic manifestations in 25% of cases (n=15), mainly confusion and photophobia; and ophthalmologic manifestations in 23% of cases (n=14), mainly myodesopsia and conjunctival injection. The main biological anomalies were elevated liver enzymes, ASAT>1.5N (84%, n=47/56), ALAT>1.5N (82%, n=47/57), a thrombopenia in 75% of cases (n=39/52), a lymphopenia (58%; n=30/52), presence of hyperbasophilic cells (56%; n=18/32), rhabdomyolysis (53%; n=23/43). An inflammatory biologic syndrome was present in all cases and there wasn’t an anomaly of renal function. An antibiotherapy by DOXYCYCLINE was prescribed in 62 % of cases (n=38). There have been no deaths in this cohort and the evolution was favorable in all cases but with a prolonged asthenia. A seasonal factor was observed with 79% of cases described between October to March (n=48), the hot season. The epidemiological data showed that most of the cases occurred in the West and South of the island, the dry zones.

Conclusion: MT is endemic on the Reunion Island and it is an emerging local disease. The results of this study are close to descriptions of MT in the literature. It is probably frequently misdiagnosed due to its low specific clinical presentation and its recent description on the island. Cases of prolonged fever (≥ 7 days) with headaches should guide to the diagnosis of TM specially in the hot season and dry zones. Elevated liver enzymes with thrombopenia are also further information to take into account when making a diagnosis. An alternative diagnosis suggested in the Reunion Island is leptospirosis which differs from TM by its almost constant renal dysfunction.
Anaplasmosis is a tickborne disease caused by the obligate intracellular Gram-negative bacterium, Anaplasma phagocytophilum. The disease often presents with nonspecific and variable symptoms and negative serology during the acute phase after a tick bite. Direct pathogen detection is the best approach for early confirmatory diagnosis. Over the years, PCR-based molecular detection methods have been developed for A. phagocytophilum, but they either suffer relatively lower sensitivity as experienced in conventional PCR or require expensive and sophisticated instruments like quantitative PCR. To improve the sensitivity and also develop an assay that can be used in resource-limited areas, we found a 171-bp sequence that has multi-copies throughout the genome of A. phagocytophilum (e.g. 16 copies in HZ strain and 19 copies in Webster strain). A Recombinase Polymerase Amplification (RPA) assay was successfully developed based on this sequence and the limit of detection was determined to be one genome copy of A. phagocytophilum. The detection of A. phagocytophilum DNA from a single bacterium could be achieved within 10 minutes of isothermal incubation at 39 degree Celsius. In addition, the amplicon sequence does not share significant homology with other species within the Anaplasma genus or genomic DNA from other genera that are phylogenetically closely related. This high specificity was demonstrated by the absence of non-specific amplification using genomic DNA from mammalian species including human and mouse or DNA from other bacteria, including Ehrlichia chaffeensis, Borrelia burgdorferi and Orientia tustusgamushi. The high sensitivity, specificity and rapidness of this assay represent a major improvement for the early diagnosis of A. phagocytophilum in human patients and for better surveillance in its reservoirs and vectors, especially in remote regions where resources are often limited.
Ehrlichia chaffeensis is an obligatory intracellular gram negative bacterium that primarily infects mononuclear phagocytes and causes human monocytotropic ehrlichiosis (HME). E. chaffeensis T1S effectors like Ankyrin (ANK) and Tandem repeat proteins (TRP) interact with diverse array of host proteins and establish a replicative niche inside the host. Recent studies have shown that the E. chaffeensis TRP120 interacts with host epigenetic machinery, which includes histone methylase NSD1, lysine specific demethylases KDM6B/JMJD3, protein components of the SWI/SNF chromatin remodeling complex ARID1B and polycomb group protein PCGF5. Polycomb group (PcG) proteins assemble in multi-subunit nuclear complexes and regulate transcriptional outcome of numerous mammalian genes. PcGs are involved in post translational modification (PTM) of histones and biochemically characterized as polycomb repressive complex 1 (PRC1) and 2 (PRC2), responsible for mono ubiquitination of H2A at lysine 119 (H2AK119Ub) and trimethylation of H3 at lysine 27 (H2K27Me3), respectively. In mammals PRCs are major repressor of Hox genes and involved in cellular proliferation and differentiation. In this study, we have determined that TRP120 translocates to the host cell nucleus early in infection and interacts with PCGF5 in the nucleus. At 48 h post infection, a dramatic redistribution of PCGFs to the ehrlichial inclusions occurs and coincides with decreased cellular levels of different PCGF isoforms. Decrease in PCGF isoforms during infection resulted in upregulation of hox gene expression, mostly from hoxB and hoxC clusters. Upregulation in hox gene expression was associated with a reduction in PRC1-mediated H2AK119Ub repressive chromatin mark. Knockdown of PCGFs with small interfering RNA (siRNA) resulted in increased ehrlichial load, demonstrating the significance of PCGF interplay in E. chaffeensis survival and replication.
Members of Chlamydiales order, such as Chlamydia trachomatis or C. pneumoniae, are human pathogens which can cause chronic infections. Apparition of chronic diseases might be due to the presence of enlarged and persistent forms named aberrant bodies (ABs). In vitro, presence of stimuli, such as β-lactam antibiotics or interferon-gamma, induces the formation of ABs. Clarke et al. have shown previously that each AB accumulates more than 16 chromosomal copies in Chlamydia trachomatis treated with penicillin, indicating that AB formation might be due to continuous DNA replication without division. However, mechanisms of ABs formation are poorly described. In order to assess the impact of diverse stress stimuli on ABs morphology and DNA replication, we used Waddlia chondrophila, an obligate intracellular bacterium known to potentially cause miscarriage in humans, as model organism. ABs morphology and potential differences in DNA content were assessed by immunofluorescence and quantitative PCR. According to their morphology and their number per host cell, two main subtypes of ABs could be defined: (i) small and multiples ABs versus (ii) large and rare ABs. DNA replication of W. chondrophila was generally not affected by the different stimuli, confirming the hypothesis that ABs are the consequence of continuous replication without division. Furthermore, the presence of distinct ABs subtypes seems to reflect different mechanisms of ABs formation. However, further studies are necessary to understand the molecular mechanisms involved in ABs formation. Indeed, the comprehension of biological mechanisms triggering the development of this persistent stage may reduce treatment failure against chronic chlamydial infections.
Rickettsial infections, caused by Orientia tsutsugamushi (scrub typhus), Rickettsia typhi (murine typhus), and members of the spotted fever group (SFG), and leptospirosis, commonly cause fever in Southeast Asia. In Vietnam, however, fewer data are available about the prevalence of these diseases. In 2015, we conducted a study in 67 patients who included fever and at least one of the following four secondary findings: eschar, skin rash, lymphadenopathy, hepatomegaly and/or splenomegaly in Quang Nam province, central Vietnam. A total of 32 patients (47.8%) were confirmed as having O. tsutsugamushi infection by either qPCR using whole blood and eschar specimens or IFA using sera. Two patients (3.0%) tested positive for R. typhi by IFA. Genetic analysis of the 56-kDa TSA gene sequences demonstrated genetic diversity of O. tsutsugamushi with 3 new genotypes (Kawasaki, TA716 and Gilliam-related) in among 4 genotypes were found. Subsequently, in 2016, another case-control study was conducted in 378 patients with acute undifferentiated fever (AUF) and 384 non-febrile patients (control group) in this province. A total of 31 patients (8.2%) were confirmed as having rickettsial infections by qPCR using whole blood and eschar specimens [19 (5.0%) O. tsutsugamushi, 2 (0.5%) R. felis, 10 (2.7%) R. typhi] in AUF group in comparison with 1 case (0.3%) of R. felis infection was found in control group. Leptospira was detected in 18 patients (4.8%) by qPCR using whole blood and urine specimens in comparison with 5 cases (1.3%) were detected by qPCR on urine in control group. Rickettsial infections and leptospirosis are underrecognized causes of acute undifferentiated fever among adults in Vietnam. This finding has implications for the local stragery of management of fever.
P1- 33) *Rickettsia* species in Italy

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*Rickettsiae* are arthropods transmitted bacteria causing rickettsiosis. This report aims to provide an updated overview of *Rickettsia* species distribution in Italy, basing on molecular studies carried out at CRABaRT, integrated with main findings obtained by other researchers. The review focus on *Rickettsia* spp. detected in Italy in humans, animals and arthropods (ticks and fleas) free-living or collected from hosts using different molecular targets. Rickettsiae detected at CRABaRT were characterized targeting *OmpA*, *ompB* and *gltA* genes and the best sequences used for phylogenetic analysis.

In humans, worth of interest is the finding of *Rickettsia* species other than the traditionally associated Spotted Fever Group Rickettsiae. Indeed, in addition to *R.conorii conorii* reports of *R.conorii israeliensis*, *R.conorii Indian tick typhus*, *R.massiliae*, *R.aeschlimannii*, *R.slovaca* occur. *Rickettsia* identifications in animals are not frequent and they include *R.conorii* in dogs, *R.slovaca* in rodents. A great variety of *Rickettsia* species was detected in ticks, especially in Southern regions where a multiform tick population exists. Main findings concerned *R.conorii conorii*, *R.conorii israeliensis*, *R.massiliae* in *R.sanguineus*, *R.aeschlimannii*, *R.slovaca* and *R.roultii* were detected in *D.marginatus*. *R.aeschlimannii* and *R.slovaca* were found in *Hyalomma* ticks. *Ixodes* ticks were positive to *R.helvetica* or *R.monacensis*. Interesting are reports of Candidatus *Rickettsia barbariae* in *R.hoogstralii* in *Hyalomma* ticks. *R.felis* was found in the flea *Ctenocephalides felis*. The study reports the occurrence of several *Rickettsia* species of public health relevance both in hosts and in vectors in Italy. Thanks to Francesca Marino. Funded by IZSSI02/13RC and IZSSI10/14RC.
Bartonella species are transmitted by animal bites and scratches or by many vectors and are responsible of human and animal diseases. The study aimed to investigate Bartonella species in asymptomatic stray cats and dogs and their ectoparasites. The analysis concerned 26 cats and 13 dogs housed in Palermo Municipality shelter (Southern Italy) and 134 fleas collected from them. EDTA blood was collected for each animal and sera for 23 cats. Fleas were morphologically identified. Antibodies against Bartonella spp. were searched by indirect immunofluorescence. DNA was extracted from EDTA blood and fleas and PCR and sequencing of Bartonella spp. 16S-23S ribosomal RNA intergenic spacer were conducted. Out of the fleas, 97 were found on cats and 37 on dogs. Ctenocephalides felis was the most prevalent species, with 132 specimens, while only two Ctenocephalides canis were collected on a dog. Two cats showed antibodies against Bartonella spp. (prevalence 8.7%). Bartonella spp. DNA was found in six animals (15.4%), 5 cats and one dog. Seventeen C.felis fleas (12.7%) were positive for Bartonella spp., 14 collected from cats and 3 from dogs. For 13 positive samples (a cat and 12 fleas) good sequences were obtained. Bartonella henselae was found in the cat and in 5 fleas, Bartonella clarridgeiae in 7 fleas. C.felis is a known vector of identified Bartonella species. Studies on stray animals and their ectoparasites are not common, thus they can be undervalued as possible zoonotic agent reservoirs. Thanks to Francesca Marino. Funded by IZSSI02/13RC and IZSSI10/14RC.
Detection of *Bartonella* spp. in *Ctenocephalides* fleas from dogs in Southern Italy

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Cat-Scratch Disease is commonly associated with *Bartonella henselae*, however new *Bartonella* species were recently associated with pathological clinical cases. This study aims to investigate the presence of *Bartonella* spp. DNA in arthropods collected from dogs in Southern Italy. The study concerned 112 ticks and 25 fleas collected from pet dogs in Sicily (Italy) in 2015. All specimens were identified by morphological keys. DNA was extracted from each arthropod and PCRs targeting 16S rRNA, 16S-23S ribosomal RNA intergenic spacer and heme-binding protein genes were performed to detect *Bartonella* spp. DNA. PCR products were visualized on agarose gel and sequenced. All ticks belonged to *Rhipicephalus sanguineus* species while the flea species *Ctenocephalides canis* (n=23) and *C.felis* (n=2) were identified. No PCR positive products were obtained from ticks, while *Bartonella* spp. DNA was amplified from a *C.felis* and a *C.canis*. Sequencing showed *B.henselae* in *C.felis* and *Bartonella rochalimae* in *C.canis*. To the best of our knowledge, this is the first report of *Bartonella* spp. in *C. canis* fleas from dogs in Italy. There are only few reports of zoonotic Bartonellae in *C.canis*. Other authors reported *Bartonella* DNA in fleas, however only *C.felis* was found positive at PCR in Italy. This result suggests to increase the epidemiological survey on *Bartonella* spp. detection in arthropods differing from the main reservoir and to investigate their role in pathogen transmission to the host. Thanks to Francesca Marino. Funded by IZSSI02/13RC and IZSSI10/14RC.
Tularemia is a zoonosis caused by Gram-negative, facultative intracellular coccobacilli belonging to the species *Francisella tularensis*. Two subspecies are responsible for animal and human infections: subsp. *tularensis* (type A strains) mainly found in North America, and subsp. *holarctica* (type B strains) found in the whole Northern hemisphere. Type A strains are classically more virulent than type B strains, the latter being separated in two main biovars: biovar I (erythromycin-susceptible) found in Western Europe and North America, and biovar II (erythromycin resistant) found in Eastern Europe and Asia. More recently, several genotypes have been defined within each of the two subspecies, using molecular markers such as PFGE profiles and canonical SNPs and INDELs. Phylogeographic studies have revealed much more complexity in the worldwide spread of these recently defined genotypes. Moreover, it has been shown that these genotypes are associated with dramatic changes in virulence, the PFGE genotype A1b being the most virulent.

The clinical manifestations of tularemia vary according to *F. tularensis* reservoirs and modes of transmission. Classically, small rodents and lagomorphs are major sources of human infections. However, the true animal reservoirs of *F. tularensis* remain poorly known. Arthropods are also probable reservoirs and highly significant sources of infections worldwide, including *Ixodidae* ticks in most endemic areas, and mosquitoes (e.g. *Aedes*) in Scandinavia. Finally, *F. tularensis* has the ability to survive for prolonged periods in the aquatic environment, possibly because of interactions with protozoa such as free amoebae. This huge reservoir is associated with a significant proportion of human infections, and more importantly is associated with large tularemia outbreaks.

The endemic areas of tularemia have been extended in the last two decades, as evidenced by major outbreaks that have occurred in Spain and Turkey. These changes are probably related to a combination of different factors, including climatic changes, the introduction by human of infected animals in non-endemic areas, and the discovery of new reservoirs. The recent demonstration that possums may transmit tularemia in humans in Australia, a continent previously considered free of tularemia, is particularly demonstrative.
The bacterial cell wall provides key structural support and shape for bacterial cells and is therefore an important antimicrobial target across a broad range of organisms. For example, Gram-negative bacteria employ lytic, cell wall-degrading enzymes to directly target neighboring competitor cells via the type VI secretion system (T6SS). Bacterial competition through the exchange of these T6S amidase effector (Tae) toxins can significantly influence the composition of polymicrobial communities. The functional importance of the Tae enzymes is further exemplified by our recent identification of tae gene acquisition by diverse eukaryotes through several independent instances of cross-kingdom horizontal gene transfer, including several vector tick species. Using a combination of biochemical and genetic approaches, we aim to understand how the horizontally acquired, domesticated amidase effector (Dae) enzyme encoded by the blacklegged tick *Ixodes scapularis* contributes to tick innate immunity by defining the mechanisms by which it controls colonization and transmission of tick-borne bacterial pathogens.
Alejandro Cabezas-Cruz – Emerging horizons for tick-borne pathogen research: from pathogen-one disease vision to the pathobiome paradigm
Mycobacterium kansasii is a member of non-tuberculosis mycobacteria. Although widespread in the environment, it is responsible of opportunistic infections in patients with underlying lung diseases or immunosuppression. In such cases, clinical presentations share many features with classical Mycobacterium tuberculosis complex infections. Seven subtypes of M. kansasii are classically defined based on hsp65 PCR and restriction fragment length polymorphism. Subtype I is by far the most frequent subtype recovered from patients and is involved in more severe clinical presentations. Conversely, subtype III is found more often to be a colonizer. Thus, in order to investigate M. kansasii virulence, we performed a comparative genomic analysis between 5 subtype I strains and 6 subtype III strains. In addition, we included in the analysis 5 subtype I and 2 subtype III publically available M. kansasii genomes as well as a reference genome for M. tuberculosis. Average nucleotide identity and core-genome analysis revealed subtype III strains to cluster into 2 different clades, unrevealing the limitations of the current classification. Orthologous predictions allowed the identification of orthologous groups specific to each subtypes: 152 and 50 for subtypes I and III, respectively. Interestingly, orthologs encoding for (i) VapBC toxin-antitoxin systems, (ii) HspX and (iii) effectors of the type VII secretion system, were found to be specific to subtype I. To conclude, subtypes definition need to be revised in the light of new genomic data. Moreover, large variations in gene content were observed between subtype I and subtype III strains and could explain their different level of virulence.
Tick cell lines are proving to be increasingly useful tools for isolation, propagation and study of intracellular bacteria harboured by ticks and other arthropods. Until recently, *Ixodes ricinus* was the only European tick species from which cell lines were available. The Tick Cell Biobank has an ongoing programme of establishing novel cell lines from additional European tick species. A network of collaborators in France, Spain, Germany and Russia have provided engorged female ticks of the ixodid species *Dermacentor marginatus*, *Dermacentor reticulatus*, *Hyalomma lusitanicum*, *Hyalomma marginatum*, *Ixodes ricinus*, *Rhipicephalus bursa*, *Rhipicephalus sanguineus* and the argasid tick *Argas reflexus*. The female ticks were surface sterilised and allowed to lay eggs; when the developing embryos were visible, the eggs were crushed to release the embryonic tissues which were then incubated at 28 °C in complete culture medium with weekly partial medium change. Tick cell multiplication may commence after weeks, months or even years *in vitro*; subcultures can then be initiated. At the time of writing, new cell lines have been established from French *R. sanguineus* and Russian *D. marginatus*, putative young lines are developing from Russian and German *D. reticulatus*, Spanish *H. lusitanicum* and German *A. reflexus*, and primary cultures set up from Spanish *H. marginatum*, *I. ricinus* and *R. bursa* have begun to grow. These novel tools will be particularly useful for research on European tick-borne bacteria of the genera *Anaplasma*, *Bartonella*, *Coxiella*, *Ehrlichia*, *Neoehrlichia*, *Rickettsia* and *Spiroplasma*, as well as viruses and protozoa.
The sheep tick *Ixodes ricinus* is the most important tick of medical and veterinary importance in Western Europe. This ectoparasite also harbours a vertically-transmitted bacterial endosymbiont, *Candidatus Midichloria mitochondrii* (Rickettsiales: Midichloriaceae), which is found in 100% of adult female and ~50% of adult male *I. ricinus* in continental Europe. The nature of the relationship between *Ca. M. mitochondrii* and *I. ricinus* is unknown, but the lifecycle of the symbiont involves replication inside mitochondria and the bacterial density increases dramatically following a blood meal. In this study, we sought to estimate the prevalence of *Ca. M. mitochondrii* in English *I. ricinus* and the population genetic structure of the symbiont in relation to its host across Europe. We assayed a total of 500 unengorged nymphs from five sites across southern England, which revealed a prevalence of *Ca. M. mitochondrii* of 80%. Although nymphs cannot be sexed easily, this prevalence estimate closely matches published data for adult ticks from continental Europe if a 1:1 sex ratio among nymphs is assumed. However, the density of *Ca. M. mitochondrii* in English nymphs was apparently higher than that previously reported from the Czech Republic. A multi-locus sequence typing (MLST) for *Ca. M. mitochondrii* was developed and used alongside an established mitochondrial MLST scheme for *I. ricinus*. New data were compared with 318 British and 188 Latvian published *I. ricinus* mitochondrial sequences that exhibited 10% and 14% variability at the DNA and amino-acid level, respectively, allowing some differentiation between British and Latvian samples. Among French, German and Italian ticks, small clusters of Italian and German mitochondrial sequences were observed, while other samples from these locations produced sequences that were distributed throughout the tree. The MLST scheme for *Ca. M. mitochondrii* indicated low levels of diversity, with some geographic clustering in continental Western Europe and partial congruence with the phylogeny of the tick host. Further co-evolutionary analyses of *Ca. M. mitochondrii* and its host will facilitate the characterisation of this symbiosis (parasitic, mutualist or commensal), as well as its origin and spread.
Stacey D. Gilk – Cholesterol and loss of the *Coxiella* intracellular niche
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*C. burnetii* is a highly infectious obligate intracellular bacterium that replicates in a specialized lysosome-like compartment called the parasitophorous vacuole (PV). PV biogenesis is a dynamic process involving vesicular trafficking and fusion events, with the PV membrane playing a central role. A distinguishing feature of the PV membrane is that it is rich in host cell-derived sterols. Inhibitor and transcriptome studies have implicated cholesterol in *C. burnetii* pathogenesis, although the role(s) of cholesterol during *C. burnetii* infection are unknown. Using live cell microscopy of infected epithelial cells, we demonstrated that fluorescent cholesterol traffics to the PV membrane. Interestingly, cholesterol supplementation of a cholesterol-free cell culture model system revealed smaller PVS and reduced *C. burnetii* growth as the cellular cholesterol concentration increased, suggesting increasing PV cholesterol is detrimental to *C. burnetii*. Surprisingly, a significant number of PVS in cells with cholesterol contained degraded bacteria, a phenotype not observed in cholesterol-free cells. Increasing PV cholesterol with U18666A, a drug that blocks cholesterol egress from lysosomes and the PV, also led to *C. burnetii* degradation. *C. burnetii*’s sensitivity to cholesterol is specific to the intracellular environment, as neither cholesterol or U18666A affected axenic bacterial cultures. To further characterize cholesterol-dependent changes in the PV, we measured fusion between the PV and host endosomes using a quantitative fluorescent dextran trafficking assay. Compared to PVS in cholesterol-free cells, PVS in cells with cholesterol were less fusogenic, and cholesterol-rich PVS containing degraded bacteria accumulated little to no fluorescent dextran. Finally, using a ratiometric microscopy-based assay with the pH-sensitive fluorophore OregonGreen488, we found that cholesterol further acidified the PV from an average of pH 5.1 to pH 4.4. Importantly, blocking PV acidification with bafilomycin recovered bacterial viability in the presence of U18666A. These data indicate that increasing PV cholesterol further acidifies the PV lumen, leading to *Coxiella* death.
Coxiella burnetii is the intracellular bacterial agent of human Q fever, an acute debilitating flu-like illness that can cause life-threatening endocarditis. Disease occurs via inhalation of contaminated aerosols, resulting in a pulmonary infection. Within host cells, Coxiella directs fusion with autophagosomes and lysosomes to generate a parasitophorous vacuole (PV) in which to replicate. We recently characterized a primary human alveolar macrophage (hAM) infection platform and now extend those studies to intact human lung tissue. Avirulent Coxiella triggered a prolonged IL-1β response in lung tissue while virulent Coxiella pathotypes did not trigger IL-1β production. Although bacteria were present in other lung cells, Coxiella only replicated within hAMs. Therefore, we used hAMs to assess the interaction of Coxiella with host autophagy, focusing on the cargo receptor p62. p62 was recruited to avirulent and virulent PV and was stabilized during infection. Additionally, p62 phosphorylation at S349 increased substantially during infection. S349 phosphorylation regulates p62 interaction with the cytoprotective Nrf2-Keap1 pathway by competing for Keap1 binding and releasing Nrf2 for translocation to the nucleus. Indeed, Nrf2 levels were stabilized during infection and the protein was present in the nucleus of infected cells. Our findings indicate Coxiella triggers Nrf2 activation in hAMs, representing a new area of investigation into the host cell oxidative response to this highly infectious pathogen.
Anne F.M. Jansen – CXCL-9, a promising biomarker in the diagnosis of chronic Q fever

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Objectives In the aftermath of the largest Q fever outbreak in the world, diagnosing the potentially lethal complication chronic Q fever remains challenging. PCR, Coxiella burnetii IgG phase I antibodies, CRP and 18F-FDG-PET/CT scan are used for diagnosis and monitoring in clinical practice. Monitoring chronic Q fever during treatment is at least equally difficult and currently, treatment decisions are based on changes in IgG phase I titers and alterations in FDG-avidity of the lesion on a PET/CT. We aimed to identify and test biomarkers in order to improve discriminative power of the diagnostic tests and monitoring of chronic Q fever.

Methods We performed a transcriptome analysis on C. burnetii stimulated PBMCs of 4 healthy controls and 6 chronic Q fever patients and identified genes that were most differentially expressed. The gene products were determined using Luminex technology in whole blood samples stimulated with heat-killed C. burnetii and in serum samples from chronic Q fever patients and control subjects.

Results Gene expression of the chemokines CXCL9, CXCL10, CXCL11 and CCL8 was strongly up-regulated in C. burnetii stimulated PBMCs of chronic Q fever patients, in contrast to healthy controls. In whole blood cultures of chronic Q fever patients, production of all four chemokines was increased upon C. burnetii stimulation, but also healthy controls and past Q fever individuals showed increased production of CXCL9, CXCL10 and CCL8. However, CXCL9 and CXCL11 production were significantly higher for chronic Q fever patients compared to past Q fever individuals. In addition, CXCL9 serum concentrations in chronic Q fever patients were higher than in past Q fever individuals.

Conclusion CXCL9 protein, measured in serum or as C. burnetii stimulated production, is a promising biomarker for the diagnosis of chronic Q fever.
Coxiella burnetii is an intracellular bacterium and the causative agent of Q fever, a disease which causes various acute and chronic presentations. The pathogenesis of C. burnetii has been characterized in many rodent models. However, these are relatively poor models of human disease as mice are significantly more resistant to infection than humans, with an infectious dose believed to be more than 4 logs higher than for man. To enable further development of therapeutics to treat Q fever and facilitate transition into man, non-human primate models are required.

Here we report the development of a non-human primate model disease using the common marmoset (Callithrix jacchus), in order to study susceptibility, dose-ranging and natural history of disease. Marmosets were challenged by the aerosol route with $10^7 - 10^5$ cfu C. burnetii Nine Mile Phase I and monitored daily post-challenge. Animals challenged with >8.7 x $10^2$ cfu exhibited a disease in marmosets that is similar to human Q fever. Infection was characterised by a resolving febrile response, with initiation and duration of fever occurring in a dose-dependent manner. This was associated with a typical weight loss between 5 and 15 days post-challenge, liver enzyme dysfunction, circulating INF-$\alpha$ and an elevated expression of CD64$^+$ (sepsis marker) on circulating neutrophils during bacteraemia. Animals were bled and euthanized at various timepoints post-challenge, with bacteraemia observed in 25% animals as early as day 3 post-challenge, peaking in all animals at day 7 and resolving by day 21. Viable C. burnetii were recovered from a variety of tissues, including the lungs of 75% animals at day 28 post-challenge, when there were no overt clinical features of disease. This is consistent with the histological evidence of macrophage and lymphocyte infiltration into the lung resulting in granulomatous alveolitis. Disease progression in the common marmoset appears to be consistent with human clinical and pathological features of Q fever, indicating that this is a suitable animal model for the investigation of novel medical interventions such as vaccines or therapeutics.
Chronic Q fever is a potentially lethal disease that can develop after an infection with *Coxiella burnetii*. Usually, it presents as an endocarditis or infection of pre-existing aneurysms and/or aortic prosthesis. We describe a fatal case of chronic Q fever, complicated by multiple immunological manifestations, such as SLE-like characteristics, cryoglobulinemia, MGUS and positive autoantibodies among which are ANA, anti-dsDNA and anti-cardiolipin IgG and IgM. Awareness of such manifestations is of major importance to avoid diagnostic delay, and these phenomena have several therapeutic consequences, in this case resulting in a false sense of adequate treatment of the infection and an unavoidable start of additional immunosuppressive therapy while still treating the ongoing infection.

To investigate the prevalence and clinical relevance of immunological manifestations in Q fever, cross-sectional studies and case reports on immunological manifestations in Q fever were reviewed. Cross-sectional studies presented specific types of autoantibodies in Q fever: cardiolipin antibodies were frequently found positive in acute Q fever patients, whereas cardiac muscle antibodies were only detected in chronic Q fever patients. Additionally, a case analysis identified multiple chronic Q fever patients with concurrent type II cryoglobulinemia. Clinical relevance of these autoantibodies and cryoglobulinemia may be substantial in terms of diagnosis and monitoring of treatment efficacy, as is illustrated by our case. In chronic Q fever, positive autoantibodies warrant further immunological investigation and cryoglobulinemia should be actively investigated as it may result in falsely negative Q fever serology.
Agathe Subtil – The type three secretion system of chlamydiae
Waddlia chondrophila belongs to the Chlamydiales order and is considered as an emerging pathogen causing adverse pregnancy outcomes in humans and abortion in ruminants. Like all chlamydiae, W. chondrophila genome encodes a complete Type 3 Secretion System (T3SS) known to secrete effector proteins. These effector proteins exhibit poor sequence homologies and they are key player in the interactions between these strict intracellular bacteria and their host cells. Several effectors have been described for classical chlamydiae, but nothing is known about effectors of Chlamydia-related bacteria. In a previous study, we have identified Wace1 as an immunogenic protein of W. chondrophila. We observed that Wace1 is secreted in the host cytoplasm. With an in silico prediction based on algorithms, we could predict that Wace1 might be secreted through T3SS with a high probability. To assess secretion by the T3SS, we succeeded in expressing Wace1 in a heterologous system, and we could observe secretion of the protein in this system. Moreover inhibition of the secretion by specific T3SS inhibitors confirmed Wace1 as the first effector protein secreted through W. chondrophila T3SS. RNA and protein expression profiles were analyzed and in accordance with a secretion of Wace1 during the early/mid phase of the infection. This timing suggests a role for this protein in establishing optimal conditions to sustain the bacteria exponential growth. Determining the interacting host cell components will provide valuable insights into the function of this putative T3SS effector and open perspectives for the development of new drugs against chlamydiae.
The increase in reported rickettsial infections globally coincides with the discovery of unfamiliar arthropod vectors, newly recognized rickettsial pathogens, and documented transmission potential of what have been considered to be rickettsial symbionts. Thus, the transmissibility of rickettsiae, vectorial capacity, and the classification of rickettsial pathogens can be considered variables contributing to emerging rickettsial infections. An emerging flea-borne rickettsiosis, caused by *Rickettsia felis*, is an example of an understudied pathogen. Multiple *R. felis* genotypes, combined with the diversity of potential vectors and transmission mechanisms, results in a unique transmission cycle with complex vector-pathogen interactions. Recent studies have identified novel aspects of transmission biology, ecology, and epidemiology of rickettsial diseases.
Tick-borne rickettsioses are zoonoses caused by spotted fever group (SFG) *Rickettsia* spp.. The incidence of the tick host and a seasonal incidence of the disease that parallels tick activity determine the geographical distribution of *Rickettsia* sp.. The first human case of infection with *Rickettsia sibirica mongolitimonae* was reported in France in 1996. This patient presented rope-like lymphangitis extending from the eschar to the draining lymph node, and *R. sibirica mongolitimonae* infection was named lymphangitis-associated rickettsiosis. Since then, mostly based on serological evidence, *R. sibirica mongolitimonae* was considered as a rare pathogen that is commonly associated with rope-like lymphangitis. Cases of *R. sibirica mongolitimonae* infection have been previously reported in patients from the Mediterranean area, in South Africa and recently in Sri Lanka and Cameroon. The last years in our laboratory, we routinely use skin biopsies and cutaneous swabs for the early diagnosis of SFG rickettsiosis. One of the advantages of this strategy is that we can easily identify the infecting rickettsial species using molecular assays. This strategy has completely modified our knowledge of the epidemiology of SFG rickettsioses in France. In a large series of patients infected with *Rickettsia* spp., *R. sibirica mongolitimonae* was the most common indigenous rickettsiosis (38%) in France, and significantly more cases occurred during the spring (April–June). The presence of a rope-like lymphangitis, an eschar on the neck and axillary lymphadenopathy, is significantly associated with *R. sibirica mongolitimonae* infection. *R. sibirica mongolitimonae* infection is a very frequent rickettsiosis, likely more frequent than even *R. conorii*, which for decades has been considered the most common *Rickettsia* sp. in this area.
Anaplasma phagocytophilum, an obligate intracellular prokaryote, infects neutrophils, reprogramming transcription and function. Long contiguous stretches of genes on neutrophil chromosomes are differentially expressed with infection, and this correlates locally with DNA methylation. Secreted A. phagocytophilum effector AnkA is a prime candidate to mediate many of the transcriptional changes since it binds in cis to and dampens transcription from gene promoters, complexes with heterochromatic DNA across all chromosomes, in part by binding to matrix attachment regions (MAR) that regulate 3D chromatin architecture. We hypothesize that identification of all AnkA binding sites will delineate aspects of how A. phagocytophilum infection results in neutrophil reprogramming. Using ChIP-seq, we showed that AnkA binds broadly and reproducibly throughout all chromosomes at: i) at promoters ≤3,000 bp upstream of transcriptional start sites; ii) intergenic regions, especially at MARs; and iii) within predicted lamina-associated domains. In RNAseq studies using ATRA-differentiated HL-60 cells that closely mimic neutrophil transcriptional programs, A. phagocytophilum infection leads to the expression of ≥ 111 alternative splicing events (ASEs) in 605 transcript-level gene identifications (18%) among significant differentially expressed transcripts. ASEs account for enrichment in 11 GO term processes, including 8 that are not identified among 53 GO processes categorized by gene level classification of differential expression, and are significantly associated with newly methylated DNA sites in A. phagocytophilum-infected neutrophil genomes. The first observation provides genome-wide evidence for AnkA as a cis regulator of gene transcription. The latter observations provide strong support for the role of AnkA as a MAR-binding protein and genome organizer, and the potential that the genome reorganization and pathogen-induced DNA methylation results in significant alternative splicing events that belie changes in specific cellular functions. AnkA is appropriately situated in the genome to promote neutrophil reprogramming and downstream neutrophil function changes, but these must be corroborated experimentally to establish a role in microbial fitness and pathogenicity.
Yasuko Rikihisa – Roles and functions of T4SS in *Ehrlichia chaffeensis*

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*Ehrlichia chaffeensis* have evolved to hijack biological compounds and processes of phagocytes and replicate inside these host defensive cells. Despite of the reductive genome evolution in this group of bacteria, genes encoding type IV secretion system (T4SS) homologous to VirB/D system of a plant pathogen, *Agrobacterium tumefaciens* have been expanded, and are highly expressed in *E. chaffeensis* in human cells. T4SS effectors: *Ehrlichia* translocated factor 1 (Etf-1, ECH0825), ECH0261, and ECH0767 are proteins unique to the family Anaplasmataceae, directly bind *E. chaffeensis* VirD4 and contain eukaryotic protein motifs and/or organelle localization signal. They are abundantly produced and secreted into the host cell cytoplasm. Etf-1 induces Rab5-regulated autophagy to provide host cytosolic nutrients required for *E. chaffeensis* proliferation. Etf-1 is also imported by host cell mitochondria where it inhibits host cell apoptosis to prolong its infection. We designed an anti-sense peptide nucleic acids (PNA) specific to Etf-1. Electroporation of *E. chaffeensis* with this PNA significantly reduced Etf-1 mRNA and protein, and ehrlichial ability to induce host cell autophagy and infect host cells. Etf-1 PNA-mediated inhibition of ehrlichial Etf-1 expression and *E. chaffeensis* infection could be intracellularly trans-complemented by ectopic expression of Etf-1-GFP in host cells. These data affirmed the critical role of Etf-1 in host cell autophagy and *E. chaffeensis* infection. Further studies on *Ehrlichia* T4SS effector molecules and cognate host cell receptors, their functions will undoubtedly advance our understanding of the complex interplay between obligatory intracellular pathogens and their hosts. Such data can be applied towards treatment, diagnosis, and control of ehrlichiosis.
Dermacentor andersoni ticks demonstrate intraspecies variation in their ability to acquire the pathogen Anaplasma marginale based on their geographic population of origin. We proposed that this differential phenotype was due the microbiome of the tick as endosymbionts have been shown to play a role in physiology, survival and acquisition and transmission of pathogens. We subsequently characterized the bacterial microbiome of two populations of D. andersoni ticks with different abilities to acquire A. marginale and showed that the microbiome was different in each population. The endosymbiont Rickettsia bellii was detected in only one of the populations. We manipulated the microbiome composition of the ticks by exposure to antibiotics and then fed the ticks on an animal that was infected with A. marginale and examined the rates and levels of pathogen uptake and correlated it with changes in the microbiome. An increase in the proportion and quantity of R. bellii in the ticks was negatively correlated with A. marginale levels. To remove confounding factors of other endosymbionts that could be contributing to the phenotype, we moved to a cell culture based system where we could introduce the endosymbiont and the pathogen. We performed competition studies examining the growth parameters of R. bellii and A. marginale in DAE tick cell culture. Both A. marginale and R. bellii grow more slowly when coinfected than when grown alone, however, this effect is more pronounced for A. marginale. Preliminary data suggests that A. marginale does not sustain an active infection in the presence of R. bellii. R. bellii may be a candidate to interfere with A. marginale tick-borne transmission through microbiome manipulation.
Infection by *Ehrlichiae* relies on the translocation of type IV secretion system virulence proteins, termed effectors, which manipulate host cell processes. However, almost few effectors are knows in theses bacteria. Here we predicted effector repertoires using a previously validated workflow called S4TE (*Searching Algorithm for Type IV Effector proteins*). This analysis identified 263 predicted type IV effectors. The effector repertoires of different *Ehrlichia* species were found to be largely, non-overlapping, and only 25 core effectors were shared by all species studied. Species-specific effectors had atypically high GC content and are in gene-sparse regions of the genome, suggesting exogenous acquisition, possibly from their hosts, vectors and other bacteria. In addition, the comparison with the *Anaplasmataceae* effector repertoires implied that some effectors might be linked to host-specificity. Furthermore, we described new protein domain combinations, which allowed the inference of as yet undescribed effectors functions. The effectors collection and network of domain architectures described here can serve as a roadmap for future studies of effector function and evolution.
Member of the Rickettsiales order, the Anaplasmataceae family contains intracellular alpha-proteobacteria and comprises the genera of Anaplasma, Ehrlichia, Neorickettsia, Wolbachia and Aegiptianella that grow within membrane-bound vacuoles of host cell origin and associated with human and animal’s infection. These infections are among the oldest known vector-borne diseases, once considered to be only veterinary pathogens as early as the beginning of the 20th century; Anaplasmataceae species have been recently recognized as emerging human pathogens in the world, then, a wide interest in studying the members of this family arose have increased dramatically. The remarkable expansion of the use of molecular well-characterized Anaplasmataceae in species infection in many geographical locations in the world has revealed the presence of a multiple Anaplasmataceae species of undetermined pathogenicity from mammals and vectors. We have developed a molecular approach for the characterization of the Anaplasmataceae bacteria including unknown species based on the genetic characterization of multiple genes family of genera specific. Our approach is based on the use of a qPCR based on the 23S RNA gene that we have previously reported to amplify the most species belonging the Anaplasmataceae family including potentially new species for the screening of all DNA samples from mammals or arthropods. Positive samples will be characterized using additional genes including 16S rRNA, 23S rRNA, groEL, and rpoB genes. Finally, the phylogenetic analyses tree based on the concatenated of multiple genes will define the correct position of the Anaplasmataceae species amplified from the DNA samples. Using this approach will have previously characterized a multiple potentially new Anaplasmataceae species infecting animals and arthropods from different geographic localization in the world. Indeed, many Anaplasmataceae species including most important human pathogens, A. phagocytophilum and E. chaffensis were firstly described as animal’s pathogens before the identification of their roles in human pathology. So, the research of yet undiscovered species from animals and arthropods is a first step for the studies the epidemiology of Anaplasmataceae in order to be prepared for the potential emergence of these species as human pathogens.
Among hematophagous arthropods, ticks transmit the greater variety of pathogens of public health and veterinary importance whose (re)-emergence is recognized worldwide. Whereas the main human and animal tick-borne pathogens are well characterised in the Northern hemisphere, very few is known concerning the diversity of tick species and tick-borne pathogens circulating within the Neotropical zone of the Americas, especially concerning the Caribbean area. Most of the epidemiological data on the topic are based on old records and focused on the main livestock pathogens such as *Ehrlichia ruminantium*, *Babesia (bovis and bigemina)* and *Anaplasma marginale*. These observations underline the need to develop high throughput diagnosis methods that will allow us to conduct large scale epidemiological surveys required to better anticipate the risk of (re)-emergence of tick-borne disease in such areas. In this context, the DOMOTICK project was designed to develop a new high-throughput real-time PCR method for a large scale screening of tick-borne pathogens potentially circulating in the Caribbean. This technology is based on high-throughput microfluidic real-time PCRs using Taqman probes (BioMarkTM dynamic arrays, Fluidigm Corporation), allowing the simultaneous detection of up to 95 pathogens across 95 samples of ticks. The choice of pathogens included in this new high-throughput technology was based on a comprehensive analysis of the literature, and on a without *a priori* detection of new or unsuspected pathogens by RNA-sequencing on nucleic acids extracted from ticks collected in Guadeloupe and Martinique. NGS analysis suggests that these ticks may harbour more pathogenic microorganisms than the currently monitored in the Caribbean, such as *Rickettsia* and *Borrelia* species of public health importance. Up to now, 40 bacterial species have been listed, including the genera *Anaplasma*, *Ehrlichia*, *Bartonella*, *Borrelia*, *Rickettsia*, *Mycoplasma*, *Francisella*, *Coxiella*, *Aegyptianella*; 14 parasites species, belonging to the genera *Babesia*, *Theileria*, *Hepatozoon*, *Leishmania*, *Rangelia vitalii*, *Cytaxxzoen felis*; and 32 arboviruses mainly belonging to viral genus of Orthobunyavirus, Phlebovirus, Nairovirus, Asfivirus, Thogotovirus, Flavivirus, Coltivirus and Orbivirus. The high-throughput real-time PCR technology developed here have been validated on tick samples collected in Guadeloupe and Martinique. Finally, this new high throughput method will allow exploratory epidemiological studies on tick-borne pathogens circulating within Caribbean ticks collected on various vertebrate hosts through some Caribbean islands, such as Trinidad and Tobago, St Kitts, Barbados, St Lucia, and Cuba, thanks to the CaribVet network, and to local veterinarians.
Anaplasmosis, cat scratch disease, and Lyme disease are emerging vector-borne infectious diseases in Republic of Korea. Although the prevalence of vector-borne pathogens in domestic animals and vector arthropods has been documented, there is very limited information about the presence of vector-borne pathogens in wild animals. Raccoon dog (Nyctereutes procyonoides) is wildlife species in East Asia and Europe, and represents a potential wildlife reservoir for zoonotic diseases. To analyze the occurrence of vector-borne pathogens in raccoon dogs, 142 carcasses (found dead) and 51 bloods (from live captured raccoon dogs) were collected from 2003 to 2010 and from 2008 to 2009 in South Korea, respectively. Additionally, 105 Haemaphysalis flava (14 larvae, 43 nymphs, 32 males, and 16 females) and 9 H. longicornis (9 female) were collected from 3 raccoon dogs. Samples of spleen and blood were examined for the presence of vector-borne pathogens DNA by nested PCR. Two A. phagocytophilum, 4 A. bovis, 2 Borrelia sp., and 2 B. henselae were detected from 193 raccoon dogs and 114 ticks. To our best knowledge, this study is the largest scale survey about raccoon dogs to analyze vector-borne pathogens from them. Moreover, the detection of A. phagocytophilum, A. bovis, B. henselae and Borrelia sp. from raccoon dogs was firstly reported in a native-habitat (East Asia) of raccoon dogs.
Tick-borne diseases have been thought to be important in Veterinary Medicine. It causes damages in various aspects from economical to public health. The wild boar (Sus scrofa) has the widest distribution of all members of the pig family and is a reservoir of various vector-borne diseases. This study was conducted to detect various tick-borne diseases from wild boars in the Republic of Korea. With the help of the Korean Wildlife Management Association, the blood and serum samples were collected from captured wild boars and they were stored at -20°C until the extraction of genomic DNA. Genomic DNA extraction was performed then polymerase chain reaction (PCR) was conducted to detect Anaplasma phagocytophilum, A. bovis, Ehrlichia chaffeensis, Bartonella spp., Borrelia spp., Rickettsia spp.. In total, 268 wild boars’ blood and serum were collected and 1 A. phagocytophilum, 1 A. bovis, 5 E. chaffeensis, 1 Borrelia spp., 1 Rickettsia spp. were detected. Genomic DNA sequencing was performed to confirm PCR results and phylogenetic analysis. As tick-borne diseases are zoonosis, they need to be investigated continuously.
ORAL SESSION 8 – JUNE 20th

Johannes Heggemann – Adhesins of *Chlamydia*
Inflammasomes represent an incredibly powerful component of the innate immune response. Essential for host defence against a range of microbial pathogens, inflammasomes regulate both pyroptosis (inflammatory cell death) and the proteolytic maturation of important inflammatory cytokines (IL-1β and IL-18). Inflammasome activation during infection can be viewed as being both beneficial and harmful as insufficient activation renders the host susceptible to infection, while inappropriate or excessive responses can result in the development of immunopathology. Murine models indicate that inflammasomes are crucial for host defence during Chlamydia sp infections, but evidence for a role in the generation of immunopathology is less clear. Despite the apparent importance of inflammasomes in host defence, mechanisms regulating inflammasome responses during Chlamydia trachomatis infection is incomplete. Additionally, emerging evidence suggests that loss of compartmentalisation and the release of immuno-stimulatory compounds (both host and pathogen) in to the cytosol, is a critical component of inflammasome activation in response to intracellular bacterial pathogens such as Chlamydia sp.

I will present recently published data that demonstrates a crucial role for STING and type-1 interferon responses in activation of inflammasomes in response to C. trachomatis infection of macrophages. I will also discuss factors that lead to inflammasome activation during infection such as; loss of compartment, effector proteins regulating inflammasome responses and the role of the non-canonical inflammasome in C. trachomatis infected epithelial cells.
Intracellular bacterial pathogens have evolved various strategies for entry into nonphagocytic cells to exploit the host environment for survival and replication. Chlamydia trachomatis is a major cause of human disease yet very little is known about the mechanisms underlying elementary body (EB) internalisation into host target cells. Adherent EBs trigger the reorganisation of the actin cytoskeleton and the associated deformation of the plasma membrane by stimulating the host Rho family GTPase Rac-1, in part through the activity of the effector protein Tarp, which is translocated into the host cell by the type III secretion system (T3SS).

We have exploited cryo-electron tomography and sub-tomogram averaging to derive the intact structure of the primordial Chlamydia trachomatis T3SS in the presence and absence of host membrane contact. Comparison of the averaged structures demonstrates a marked compaction of the basal body (4nm) occurs when the needle tip contacts the host cell membrane. This compaction is coupled to a stabilisation of the cytosolic sorting platform-ATPase complex. These findings reveal the first structure of a bacterial T3SS engaged with a eukaryotic host, and reveal striking ‘pump-action’ conformational changes that underpin effector injection. Cryo-electron tomography of entry foci demonstrated that several morphologically distinct host cell structures are observed at EB entry sites. We have applied inhibitor-based screening and employed reporters to systematically assay and visualise the spatio-temporal contribution of diverse endocytic signalling mediators to C. trachomatis entry.

In addition to the recognised roles of the Rac1 GTPase and its associated nucleation-promoting factor (NPF) WAVE, our data have revealed an additional unrecognised pathway sharing key hallmarks of macropinocytosis, namely: i) amiloride sensitivity, ii) fluid-phase uptake, iii) recruitment and activity of the NPF N-WASP, and iv) the localised generation of phosphoinositide-3-phosphate (PI3P) species. Given their central role in macropinocytosis and affinity for PI3P, we assessed the role of SNX-PX-BAR family proteins. Strikingly, SNX9 was specifically and transiently enriched at C. trachomatis entry foci. SNX9-/- cells exhibited a 20% defect in EB entry, which was enhanced to 60% when the cells were infected without sedimentation-induced EB adhesion, consistent with a defect in initial EB-host interaction. Correspondingly, filopodial capture of C. trachomatis EBs was specifically attenuated in SNX9-/- cells, implicating SNX9 as a central host mediator of filopodial capture early during chlamydial entry. Our findings identify an unanticipated complexity of signalling underpinning cell entry by this major human pathogen, and suggest intriguing parallels with viral entry mechanisms.
Denys Pogoryelov – *Chlamydia energy* metabolism revisited: V/A-ATPase in focus as a novel drug target

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*Chlamydia* species are the obligate intracellular parasites for which improper treatment develops in chronic infections and associated with multiple diseases. Chlamydial cells largely depend on the uptake of ATP from their hosts. However, the path of autonomous supply of ATP in the chlamydial cells is uncertain at the circumstances when ATP scavenging from the host cells is impeded.

The omics analysis indicate that chlamydial cells produce several bioenergetics protein complexes that may be involved in the regeneration of ATP autonomously by chlamydial cells via rudimentary respiratory chain. However, this hypothesis, up to now, lacks experimental verification. We aim to revise the processes of energy metabolism in *Chlamydia* cells, particularly by studying the functional role of putative Na⁺ transporting respiratory chain complexes and associated with it components towards the deeper understanding of chlamydial energetics and assessment of these components as novel targets for anti-chlamydial therapy in humans. We set to address chlamydial V/A-type ATPase as the putative drug target. Since no specific and medically relevant inhibitors of this type Na⁺/H⁺-V/A-ATPase are currently available, for validation of this target, we are in the process of designing new effective compounds by chemical ligand libraries screening and structure-based *in silico* docking pipelines.

Our current progress on the functional and structural characterisation of the chlamydial V/A-ATPase will be presented at the meeting.
Marie de Barsy – Identification of *Waddlia chondrophila* proteins involved in transcriptional regulation

Marie de Barsy1, Lucas Hergot1, Patrick Viollier2 and Gilbert Greub1

Waddlia chondrophila is an emerging pathogen belonging to the Chlamydiales order responsible for abortion in bovine and miscarriage in human. Chlamydiales order includes nine different families; the most studied one being the Chlamydiaceae since it includes important human pathogens such as Chlamydia trachomatis and Chlamydia pneumoniae. Transcriptional regulation is an essential process for all living organisms. In bacteria, it is achieved at three different levels, (i) the RNA polymerase level (sigma factors...), (ii) the promoter level (transcription factors) and (iii) the DNA structure level (nucleoid-associated protein, histone-like proteins...). Sigma factors and DNA supercoiling are well studied in Chlamydiaceae while conserved transcription factors and their regulatory networks have recently been studied among Chlamydiae phylum.

Here, we propose to identify new factors involved in transcriptional regulation using heparin chromatography followed by mass spectrometry. With this original approach, we could identify nine proteins from the soluble fraction of purified W. chondrophila. Among them, eight were confirmed individually by heparin chromatography. We raised antibodies against three of them and performed ChIPseq analysis. Two of them showed a similar pattern, with 617 shared peaks, suggesting that these two proteins may form a complex. The third one, harbour a typical pattern of a chromatin associated protein, i.e. a binding along all the genome. Currently, we are investigating their specific role in the transcriptional regulation of W. chondrophila.

The identification of new transcription factors and their regulon will improve our understanding of the global transcriptional regulation of chlamydiae and could be used as new drug targets.
Waddlia chondrophila is an intracellular obligate bacterium, member of the Chlamydiales order and known as a causative agent of abortion in ruminants and miscarriage in humans. The division of Chlamydiales is conserved among its members and differs from the classical division machinery by lacking the homologue of the bacteria division organizer FtsZ and certain division proteins homologues. Another specificity of the Chlamydiales order is an atypical peptidoglycan, which is required for chlamydial division. We recently described septal proteins, MreB, RodZ and NlpD, which play important roles in cell division and in peptidoglycan remodeling in W. chondrophila. To better characterize the composition of the chlamydial division machinery, interactors of RodZ were investigated by Yeast-two-hybrid assay. Several promising putative interactors of the division septum protein RodZ and peptidoglycan were selected. We investigated expression and localization of the interactor candidates by immunofluorescence and quantitative RT-PCR. Furthermore, heterologous overexpression of these candidate interactors in E. coli in stringent conditions reveals impaired bacterial growth and partial proliferation inhibition, along with morphology defects, indicating that these proteins might play a role in bacterial proliferation. More work will help us to better characterize the exact role of these proteins in chlamydial division and in peptidoglycan remodelling.
Jean-Lou Marié – Epidemiology of infections caused by Anaplasmataceae in animals

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The Anaplasmataceae family, order Rickettsiales, gathers together obligate intracellular alpha-proteobacteria. Among the main genera of this family, Anaplasma, Ehrlichia and Neorickettsia species grow within membrane-bound vacuoles of vertebrate host cells while Wolbachia includes numerous species endosymbionts of arthropods and filarial nematodes. Some of these agents are among the oldest known vector-borne diseases. *Ixodes* ticks but also *Rhipicephalus, Dermacentor, and Amblyomma* genera of ticks act as vectors for the transmission to vertebrate animals and humans. A transstadial transmission exists in ticks but not a transovarial one. Therefore each tick generation has to acquire infection by feeding on a reservoir host, generally a wild mammal, thus allowing the maintenance of the bacteria in nature.

Representatives of this family were historically first responsible for animal diseases, such as *Anaplasma phagocytophilum* the etiological agent of tick-borne fever in sheep already reported in Norway in 1780 or *Ehrlichia canis* responsible for the monocytic canine ehrlichiosis described in Algeria in 1935. More recently members of this family have been recognized as emerging human pathogens and among them emerging ones. *Candidatus Neoehrlichia mikurensis* is now considered as a significant public health agent in Europe. Furthermore, some species that were considered non-pathogenic for decades are now associated with human infections.

New Anaplasmataceae species continue to be detected or isolated from mammals and ticks around the world, in tropical and temperate countries, relying mainly on the advances in molecular detection tools. This extensive research work is necessary to better understand the complex epidemiology of the life cycle of Anaplasmataceae bacteria including interactions between the environment, tick vectors, animal reservoirs and susceptible animal species and humans.
Genetic diversity of the tick-borne pathogen *Anaplasma phagocytophilum* is very high in the west, where no single rodent host serves as an outstanding reservoir. Rather, the pathogen variants circulate in a diversity of small mammals. In other geographical regions, simpler phylogenetic trees and reservoir host ecologies may indicate more recent introduction or better co-adaptation between pathogen and hosts. Here I explore broad evolutionary and ecological patterns of the origin and spread of this pathogen including the possibility that its association with rodents is a derived character with important implications for human risk.
José Antonio Oteo – *Candidatus* Neoehrlichia mikurensis in Europe

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`Ca. N. mikurensis` is a small gram negative pleomorphic cocci bacteria, included in the α-1 proteobacteria, that lives in an interacting cycle affecting rodents and ticks. It grows in a membrane-bound inclusion within the cytoplasm of endothelial cells. The specific mechanism of infection is unknown. Not cultivated yet, provisionally it has been included in the family Anaplasmataceae.

The vector in Europe is *I. ricinus*. `Ca. N. mikurensis` been detected in at least 20 countries from Europe in *I. ricinus* collected from vegetation, rodents, hedgehogs, sheep, wild board, cattle, birds or humans. It has been also detected in *Dermacentor reticulatus* and in other *Ixodes* spp. The reservoir are different types of rodents. `Ca. N. mikurensis` has been also detected in dogs, hedgehogs, badgers, chamois, mouflons and humans.

The first implication as human pathogen in Europe was made in 2010 in a patient from Sweden affected of a chronic lymphocytic leukemia. Since this description, it has been published 16 symptomatic patients (9 Sweden; 2 Germany; 2 Czech Republic, 3 Switzerland). Most patients (75%) was affected by hematologic neoplasms or under immunosuppressive conditions. Although the incubation period is unknown, a previous tick bite was referred by 56% of patients. Most of them had prolonged fever, arthralgia and myalgia. Half of patients had vascular/thromboembolic events and 37% erysipela-like/erythema nodosum lesions. A big delay in the diagnosis was the rule. `Ca. N. mikurensis` has been detected in blood of 4 patients from The Netherlands affected by erythema migrans and in asymptomatic people bitten by ticks from Sweden, The Netherlands and Poland.

The microbiological diagnose is based on molecular assays (PCR-RTPCR (16S rRNA or GroEL)). There are no serological assays for detecting antibodies. No cross-reactions with other members of the Anaplasmataceae family has been described.
In a few European countries a new pathogen transmitted by ticks Ixodes ricinus - Candidatus Neoehrlichia mikurensis was identified. Worldwide 23 cases were described, among which 16 in Europe. In Poland infection was confirmed in 5 forestry men. Due to the fact that the Ixodes ricinus ticks are common in Poland, there is a probability of infection with Candidatus Neoehrlichia mikurensis in humans. Therefore we aimed to search for Candidatus Neoehrlichia mikurensis, as an etiological factor of non-specific symptoms after tick bite.

Blood samples of 665 patients with various symptoms after tick bite were examined for Candidatus Neoehrlichia mikurensis DNA presence. Molecular detection of Candidatus Neoehrlichia mikurensis species was performed by NESTED PCR - amplification of the a specific DNA sequence of a groESL was executed (Richter et al. 2012). Positive control was acquired from Center for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, Kalmar, Sweden.

In 3 out of 665 patients (0.4%) Candidatus Neoehrlichia mikurensis DNA was detected. All patients had fever, headache, nausea or vomits, muscle pain, meningeal signs. In all patients meningitis was excluded based on cerebrospinal fluid examination.

The possibility of human infection Candidatus Neoehrlichia mikurensis in north-eastern Poland was shown, confirming the necessity to conduct research on a larger scale. Moreover, awareness of physicians about the possibility of infection in patients with various symptoms after tick bite should be increased.
Norio Ohashi – *Anaplasma phagocytophilum* in Japan

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*Anaplasma phagocytophilum* causes human granulocytic anaplasmosis (HGA) that is a tick-borne and febrile emerging infectious disease. In Japan, the surveillance of *Rickettsiales* bacteria, especially human pathogens including *A. phagocytophilum*, has conducted and continued for many years in several laboratories. To date, our survey revealed that *A. phagocytophilum* is likely transmitted by 6 tick species as arthropod vector candidates such as *Haemaphysalis formosensis*, *H. longicornis*, *H. megaspinosa*, *Amblyomma testudinarium*, *Ixodes ovatus*, and *I. persulcatus*. In recent years, HGA patients have been discovered in Japan, especially it seems to be endemic in western and central parts of Japan. We realized that it is hard to detect an antibody against *A. phagocytophilum* in sera from Japanese patients for HGA sero-diagnosis. In indirect immunofluorescence assay, the patients’ sera were reactive with antigens of *A. phagocytophilum* cultured in THP-1 cells rather than in HL60 cells. *A. phagocytophilum* has p44/msp2 multigene encoding multiple P44 major surface protein antigen species that are similar, but not identical, to each other, and generates antigenic variation. We found that *A. phagocytophilum* expresses P44-47E and P44-60 transcripts in THP-1, but it may produce P44-18ES in HL60 cells, and the sera from HGA patients in Japan allow to react with either those 3 P44 recombinant protein antigens. These findings suggest that the 3 rP44 recombinant proteins are useful for HGA sero-diagnosis as antigens in Japan.
The aim of this study is to investigate the prevalence of tick-borne pathogens in large dog breeds. Canine bloods were collected from 103 dogs consist of 42 Belgian Malinois, 58 German Shepherd, and 3 Labrador Retriever in Gangwon province (Korea) from June to October 2016. Polymerase chain reaction (PCR) was performed to detect several pathogens such as severe fever with thrombocytopenia syndrome virus (SFTSV), Anaplasma phagocytophilum, A. bovis, Ehrlichia canis, E. chaffeensis, Bartonella spp., and Borrelia burgdorferi. To isolate SFTSV from positive canine serum, Vero cells were used. For identification of isolated virus, the SFTSV S, M, and L segment genes were amplified using RT-PCR. Additionally, indirect immunofluorescent antibody assay (IFA) was performed for confirming SFTSV. SFTSV was detected in 3 (2.9%) samples using RT-nested PCR targeting the S segment. Of the three SFTSV positive sera, the SFTSV was isolated from one positive serum. Using Vero cell infected with the isolated virus, IFA was conducted. Until 1,600-fold dilution, infected Vero cell was positive against SFTSV antibody. A. phagocytophilum was detected in 2 (1.9%) samples using nested PCR targeting the 16S rRNA and other pathogens were not detected. This study is the first report of SFTSV isolation from dogs in Korea and represents that domestic large dog breeds are exposed to tick-borne pathogens.
P2.1) Amira Nebbak – Detection of bacteria in *Ixodes ricinus* ticks in the Alsace region, France

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Fifty *Ixodes ricinus* ticks collected in the region of Murbach (Haut-Rhin, Alsace, France) were tested by real-time and conventional PCR for detection of tick-associated bacteria and protozoan parasites. In total, 70.0% (35/50) of ticks contained at least one microorganism species among which 25.7% (9/35) contained 2 or more species.

Several human pathogens were identified in ticks including *Borrelia burgdorferi* s.s. (6%), *Borrelia garinii* (2%), *Borrelia valaisiana* (4%), *Borrelia miyamotii* (2%), *Rickettsia helvetica* (6%), *Bartonella* spp. (8%), *Babesia* spp. (2%) and a *Wolbachia* spp. (8%) were also detected. No evidence of *Coxiella burnetii, Anaplasma phagocytophilum* or *Bartonella henselae* carriage was found. The most common co-infections involved *Anaplasmataceae* and *Bartonella* spp. (6%), *Anaplasmataceae* and *Rickettsia* spp. (6%), and *Borrelia* spp. and *Anaplasmataceae* (4%). Co-infection involving three bacteria was seen between *Anaplasmataceae, Borrelia* spp. and *Bartonella* spp. (2%). These results highlight the panel of infectious agents that may be transmitted by *Ixodes ricinus* nymphs to humans and animals. Co-infection with at least 2 pathogens was observed in the present study suggesting the risk of the transmission of more than one pathogen during the tick blood feeding.
Brucellosis is considered to be the most common bacterial zoonosis in the world, with more than half a million new cases estimated each year. Despite control/eradication programs, brucellosis, a major worldwide zoonosis due to the *Brucella* genus, is endemic in Northern Africa and remains a major public health problem in Algeria, where human brucellosis is ranked second in notifiable zoonotic diseases. This study summarizes official data, regarding human brucellosis incidence in Medea, from 2004 to 2014, with the purpose to provide appropriate insights concerning the epidemiological situation of human brucellosis in this area. Data were obtained from the public health service of Medea. In total, 884 (349 female and 535 male) cases of human brucellosis were recorded during 10 years in Medea. The most affected age group is between 20 and 24 years old with 357 cases, followed by the age group between 45 and 65 years with 128 cases. The number of brucellosis human cases reported here, highlights the impact of the animal reservoir on the public health. Human incidence might reflect the true epidemiological situation of brucellosis in animals. The transmission of *Brucella* infection to humans in Algeria depends largely on the animal reservoir and several factors like food habits, methods of processing milk and milk products, social customs, climatic conditions, socioeconomic status, husbandry practices and environment hygiene. Collaboration between the department of health and department of veterinary services is important for the control of brucellosis in animals and thereby eliminate transmission to humans. Training of livestock farmers on the effective implementation of sanitary and hygienic livestock management practice helps to reduce spreading of the disease among animals as well as humans.
Ticks of the group *Rhipicephalus sanguineus* s.l. are major vectors of pathogens to dogs and to human and have a worldwide distribution. Previous phylogenetic reconstructions suggested the existence of two main lineages within this group, quoted “Tropical” and “Temperate”. Symbiotic interactions remain of main interest as several studies assessed their contributions to vectors development, survival, reproduction and the vector competence. However, the diversity of microbial communities within *R. sanguineus* s.l. across different populations remains poorly explored. Consequently, this knowledge is essential for future studies on hosts - symbionts - pathogens interactions. To get further insight into the bacterial communities associated with *R. sanguineus* s.l. ticks, 40 specimens from France, Senegal and Arizona were analyzed by high throughput 16S amplicon sequencing. All the specimens were genetically characterized by haplotyping 12S rDNA fragment in order to overcome any misinterpretation linked to the controversial taxonomic status of *R. sanguineus*. All *R. sanguineus* s.l. from Senegal and France were affiliated to the “Tropical” and “Temperate” lineages respectively whereas both lineages were detected in Arizona. Regardless of the origin of ticks, each bacterial microbiota was dominated by three genera, *Coxiella*, *Rickettsia* and *Bacillus*. Females harbored a microbiota dominated by *Rickettsia* or *Coxiella* whereas males had higher proportion of *Bacillus*. Significant differences of relative abundances were evidenced between specimens from different geographical origins. Our results suggest a difference in the microbiota structure according to *R. sanguineus* s.l. genotypes, geographical origin and gender. These results constitute basics for future prospects on the symbiotic interactions, biology and vector competence within the *R. sanguineus* complex.
P2-4) Sonia Santibanez – Isolation of Rickettsia amblyommatis in HUVEC cell line

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Rickettsia amblyommatis, formerly named Rickettsia amblyommii and Candidatus Rickettsia amblyommii, is an intracellular bacterium belonging to the Spotted Fever Group Rickettsia (SFGR). It is highly prevalent in A. americanum and in other Amblyomma spp. through the Western Hemisphere. R. amblyommatis has been cultivated in chicken fibroblast, primary embryonated chicken eggs, Vero cells, ISE6 tick cells and AAE2 tick cells. Due to the affinity of rickettsiae to invade vascular endothelial cells we tried to isolate R. amblyommatis from a nymph of Amblyomma cajennense sensu lato collected in Saltillo (Coahulia - Mexico) using Human Umbilical Vein Endothelial cells (HUVEC). One half was positive for R. amblyommatis by ompA PCR. The other half was selected for in vitro culture of Rickettsia spp. It was triturated in 1 ml of endothelial cell growth medium with 1% antibiotic-antimycotic solution, and the homogenate was inoculated into a HUVEC cell line. Culture was maintained at 33ºC in endothelial cell growth medium plus 2mM L-glutamine and 2% fetal calf serum, with 5% CO₂. The medium was changed weekly. Culture was checked by Gimenez stain for Rickettsia-like intracellular organisms. After 48 days of incubation, Rickettsia-like were observed in HUVEC. PCR assays and sequencing of ompA in the culture suspension showed 100% identity with R. amblyommatis. This isolate was successfully established in HUVEC cells, and it has been deposited in the collection of the Center of Rickettsioses and Arthropod-Borne Diseases, Infectious Diseases Department, Hospital San Pedro-CIBIR. The HUVEC cell line is a useful tool for the isolation of R. amblyommatis.
The closely related species *Rickettsia conorii* and *R. africae* are both etiological agents of rickettsiosis, a tick-borne serious infective disease. The laboratory diagnosis is mainly based on serological methods. However, this technique requires the production of rickettsial antigens, which is burdensome. Moreover, whole cell serology remains not enough specific to provide the diagnosis at the species level. Here, we attempted to identify specific proteins that would enable the discrimination of *R. africae* sp from *R. conorii* sp infections. We screened 22 *R. africae*- and 24 *R. conorii*-infected sera at different course of infection using a traditional immunoproteomic approach. In parallel, we focused on the technical development of a “relatively new technique” named a proximity ligation assay coupled to two-dimensional Western blotting (PLA WB). We proposed several proteins discriminating these two rickettsial species at early and active stages of infection. The top range markers of *R. africae* early infection were rpoA, atpD, and acnA, *R. africae* active infection were rOmpB β-peptide, OmpA, groEL and ORF1174, early *R. conorii* infection was prsA, *R. conorii* active infection were ftsZ and cycM. Our results suggest that top range markers are most likely candidate antigens for serodiagnosis of rickettsioses.
The primary vectors of Rickettsia rickettsii in the United States include Dermacentor andersoni, Dermacentor variabilis and Rhipicephalus sanguineus. In addition, Amblyomma americanum has been identified as a competent vector of Rickettsia rickettsii under laboratory conditions. The geographical range of A. americanum overlaps with the distribution of Dermacentor variabilis and different stages of these ticks often parasitize the same vertebrate hosts, permitting interspecies spillovers of pathogens. The current study aimed to assess the biological effects of R. rickettsii infection in A. americanum by comparing parameters of feeding, molting, longevity, fertility and fecundity of R. rickettsii infected ticks to those in uninfected A. americanum. R. rickettsii infected A. americanum larvae fed faster than uninfected larvae and a slightly greater percentage successfully molted to nymphs. There was not a statistical difference in the nymphal feeding duration, feeding success or molting success between infected and uninfected nymphs. R. rickettsii infected A. americanum nymphs survived for a longer period of time without feeding than the uninfected nymphs. R. rickettsii infected females fed to repletion normally and produced viable progeny. Overall, R. rickettsii infection did not cause apparent deleterious effects on the survival and propagation of A. americanum under laboratory conditions.
It is generally presumed that 24-48 hours from the moment of tick attachment required for reactivation of Rickettsia inside the tick before it is transmitted to the host. To elucidate the length of reactivation period, we exposed guinea pigs to bites of R. rickettsii-infected Dermacentor variabilis and allowed ticks to remain attached for 0 to 48 hours. Following removal of attached ticks, salivary glands were immediately tested by PCR, while guinea pigs were observed for 10-11 days post-exposure. Animals were then necropsied and samples of internal organs were tested by PCR for confirmation of disseminated infection. Guinea pigs in a control group were subcutaneously inoculated with salivary glands from unfed D. variabilis from the same cohort. Surprisingly, a few animals in every exposure group developed clinical and pathological signs of infection. The severity of rickettsial infection in animals increased with the length of tick attachment. However, even attachments for less than 8 hours resulted in clinically identifiable infection in some guinea pigs. Several of the control guinea pigs also developed signs of disseminated rickettsial infection. These findings suggest that the previously reported delay in horizontal transmission of Rickettsia by infected ticks is related to the quantity of the pathogen being delivered by ticks during feeding rather than required "reactivation period". Results of our study also indicate that R. rickettsii present in unfed ticks does not necessarily require a reactivation period and infected ticks are capable of transmitting infectious pathogen virtually as soon as they attach to the host.
P2-8) Katarina Stefanidesova – The effect of Thyphus serpyllum and Citrus bergamia essential oils on the growth of Rickettsia slovaca and Rickettsia conorii caspia in Vero cell line

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The aim of this study was to evaluate the impact of two essential oils (EOs) - bergamot (Citrus bergamia) and wild thyme (Thymus serpyllum) on growth curves of Rickettsia slovaca and R. conorii caspia. R. slovaca-infected monolayers cultivated with 0.01 % (dose non-cytotoxic for Vero cells) thyme EO seemed unaffected even on the fourth day post infection (p.i.), whereas cytopathic effect (CPE) was observed in controls and bergamot treated cells since the second day p.i. CPE was not visible in R. conorii infected cells. However, rickettsiae were visualized by microscopy. Thyme EO significantly decreased growth of R. slovaca to 2.7, 0.23, and 0.02 % of mean number assessed in controls by qPCR; and 1.44, 0.09 and 0.03 % by RT-qPCR on days 2, 3 and 4 p.i., respectively. Average number of R. conorii particles in thyme treated cells estimated by qPCR equaled to 1.18, 0.01, and 0.01 %; and by RT-qPCR 7.8, 0.57, and 0.19 % of controls. R. conorii load in bergamot-treated cells was 26.47, 5.42, and 3.04 % of those in controls estimated by qPCR; and 36.87, 19.36, and 21.22 % by RT-qPCR. Our results showed that EOs e.g. thyme may significantly inhibit the growth of rickettsiae. Further studies are necessary to elucidate rickettsicidal properties of EOs, and possible implications for their use in acaricide, repellent or prophylactic products. Acknowledgements: This study was financially supported by the projects Vega Nos. 2/0106/16, 2/0068/17, SRDA-0280-12, International Visegrad Fund 21610493.
Rickettsialpox is a disease caused by *Rickettsia akari*. Cases of this underestimated rickettsial disease have been described in diverse parts of Europe and American continents. The information on immunogenicity of *R. akari* is limited and this can lead to a weak diagnosis or under recognition of the disease. The aim of this study is to investigate the protein profile of *R. akari* and study the antigenicity of its proteins using one (1D) and two dimensional gel electrophoresis (2D) and immunoblotting techniques. Our results showed positive signals from 6 kDa to 200 kDa in 1D western blot. 2D protein gel staining by Coomasie blue confirmed the presence of proteins ranging between 130 and 10 kDa and pI interval from 3 to 10. The corresponding 2D immunoblot revealed the known ones rickettsial immunogenic proteins: OmpB, 60 KDa chaperonin (GroEL) and Elongation factor Tu (uniprot: Q92GW4). In addition, we were able to detect three more specific immunogenic proteins for *R. akari*, i.e. Peptidoglycan-associated lipoprotein (uniprot A8GPW0_RICAH), 30S ribosomal protein S12 (uniprot: A8GM98RS12_RICAH) and Periplasmic serine protease (A8GM89_RICAH). These results can contribute to the improvement of Rickettsialpox diagnosis.

**Acknowledgements:** This study was partly supported by grant agencies: VEGA: 2/0139/16, VEGA: 2/0173/15, and APVV-0280-12.
Rickettsiae may cause an unbridged variety of diseases with neurological manifestation, which may have consequences for etiopathogenesis of serious neurological diseases. In this study, we engaged an experimental model of rat embryonal and in vitro differentiated cerebrocortical neurons for investigation of direct effect of rickettsiae on the neurons. Microscopic visualization revealed intraneuronal localization of investigated Rickettsia species. We found a strong reduction in viability of rat cerebrocortical neurons in vitro, which was associated with significant activation of apoptosis related and proinflammatory molecules. Our data further clarify molecular mechanisms, which are involved in rickettsiae mediated neurotoxicity.

Acknowledgements. This study was financially supported by the projects VEGA 2/0005/15 from the Scientific Grant Agency of Ministry of Education and SAS, APVV-0280-12 and APVV-0677-12 from Slovak Research and Development Agency.
Background: Autoimmune diseases are characterized by an adaptive immune response of T- and B-cells directed against self-antigens. Infectious diseases are thought to be one of the major triggers of autoimmunity in genetically predisposed individuals. Cryoglobulinemia is the precipitation of immunoglobulins at low temperatures, which may occur secondary to an infection. Chronic Q fever is a persistent infection with the intracellular Gram-negative bacterium Coxiella burnetii which predisposes to aneurysms, aortic prosthesis or heart valves. Due to the absence of specific symptoms and presence of concomitant, and sometimes symptomatic, immunological phenomena, diagnosis of chronic Q fever can easily be delayed or even missed. Here, we aim to identify autoimmune phenomena associated with chronic Q fever.

Methods: We collected data on reported autoimmune diseases and immunological parameters including auto-antibodies and cryoglobulin from patients with proven chronic Q fever, who were treated at the outpatient clinic of the Radboud university medical center (Radboudumc) in Nijmegen, the Netherlands.

Results: Immunological parameters were collected of 39 chronic Q fever patients, of which 20 were positive for autoantibodies. Anti-nuclear antibodies (n=35) were present in 37% of patients and anticardiolipin IgM and IgG (n=33) were present in 3% and 6% respectively. Other reported auto-antibodies were smooth muscle antibodies (20% of 20 patients) and anti-neutrophil cytoplasmic antibodies (16% of 19 patients). During treatment or shortly before chronic Q fever diagnosis, two patients were diagnosed with systemic sclerosis, two patients had SLE-like symptoms, and one patient developed both a thyroiditis and myocarditis. All these patients required immunosuppressives. Cryoglobulin, determined in 30 patients, was detected in 8 patients and most commonly consisted of a mixed type III cryoglobulinemia. In one patient, C. burnetii IgG phase I antibodies were nearly undetectable in serum during follow-up, indicating a beneficial response to therapy. Upon revision, the cryoprecipitate contained complexes of these C. burnetii IgG phase I antibodies in abundance and antibiotic therapy was intensified.

Conclusions: Autoantibodies, autoimmune syndromes and cryoglobulinemia were frequently present in this cohort of chronic Q fever cases. These findings have important implications in clinical practice, as they may delay diagnosis, hamper adequate monitoring of treatment effect and necessitate immunosuppressive treatment.
P2-12) Sonja E. Van Roeden – Serum doxycycline concentration-based dosing in the treatment of chronic Q fever

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First choice treatment for chronic Q fever is doxycycline plus hydroxychloroquine. Serum doxycycline concentrations (SDC) >5µg/mL were associated with favorable serological response. To study the effect of measuring SDC in practice on clinical outcome, we performed a retrospective cohort study in proven or probable chronic Q fever patients treated with doxycycline and hydroxychloroquine ≥12 weeks. Primary outcome was the first disease-related event (new complication / chronic Q fever-related mortality); secondary outcomes were all-cause mortality and PCR-positivity. Multivariable analysis was performed with a Cox regression model. To adjust for intensity of patient care, the ratio of number phase I IgG antibody titer measurements and follow-up duration was used as a proxy. We included 201 patients: in 167 (83%) SDC were measured (mean age 68 years, 83% male). SDC measurement was associated with a lower risk for disease-related events (HR0.50, 95%CI 0.26-0.96, p=0.03), but not with all-cause mortality or PCR-positivity. Adjustment for intensity of patient care did not change the estimates. First SDC was >5 µg/mL in 106 patients (63%), all with 200 mg doxycycline daily. In patients with SDC measured, dosage was adjusted in 41% (n=68). Overall mean of measured SDC was >5 µg/mL, in patients with and without primary and secondary outcomes, indicating titration of dosage based on SDC. Our results suggest that SDC measurement decreases the risk for disease-related events, potentially through more optimal dosing or through improved compliance. We recommend measurement of SDC and titration of doxycycline dosage to SDC >5 µg/mL during treatment of chronic Q fever.
Coxiella burnetii, the etiologic agent of Q fever, replicates in an intracellular compartment with low pH. The impact of this low pH environment on antimicrobial treatment is not well understood. An *in vitro* system for testing antibiotic susceptibility of *C. burnetii* in axenic media was set up to evaluate the impact of pH on 1) *C. burnetii* growth and survival, and 2) the efficacy of doxycycline and rifampin. The Nine Mile Phase 2 strain of *C. burnetii* in log phase was diluted into fresh flasks of ACCM-2 media with varying pH and antibiotic dosage.

Cultures were tested by qPCR for the quantity of *C. burnetii* at the start of the culture and after 7 days' incubation. After incubation, an aliquot of the culture was diluted into fresh ACCM-2, pH 4.75 and grown for 1 week to evaluate viability. In this system, increasing pH above 5.75 was bacteriostatic for *C. burnetii*. At pH 5.25 and 4.75, doxycycline and rifampin had bacteriostatic activity against *C. burnetii* at very low doses (0.1 and 0.01 ug/ml) and were bactericidal at a higher dose (1 ug/ml). At pH 6.25, rifampin retained bactericidal activity, but doxycycline was bacteriostatic. Changing pH from 4.75 to 5.25 improved the efficacy of doxycycline. The data show that doxycycline and rifampin are effective at low doses in the ACCM-2 system, and they both can be bactericidal against *C. burnetii*. The bacteriostatic activity of doxycycline at higher pH suggests that doxycycline may only be bactericidal under pH conditions where *C. burnetii* can replicate.
Background: Chronic Q fever is caused by a persistent infection with *Coxiella burnetii* of an aneurysm, vascular prosthesis or heart valve. It is thought that elimination of the infection requires Interferon-gamma (IFN-γ), either derived from T-cells or NK-cells. *In-vitro* administration of IFN-γ to *C. burnetii* infected monocytes reduces bacterial viability. IFN-γ has been successfully administered to two young children described in literature, one with multifocal osteomyelitis and one with intractable fever that did not respond to antibiotic therapy.

Methods: We report a 75-year-old man with proven chronic Q fever based on high IgG anti-*C. burnetii* phase I antibodies (1:16384) and an infected aortic prosthesis complicated by a PCR positive *C. burnetii* psoas abscess. He received multiple antibiotic regimens: doxycycline with blood levels above 5 mg/L with hydroxychloroquine for 2 years (stopped because of hyperpigmentation and clinical failure), followed by moxifloxacin, cotrimoxazole and eventually claritromycine. Because of clinical failure, a trial with IFN-γ injections was initiated in addition to claritromycine. Before and during treatment, the patient was monitored cautiously by serological titre measurement, *in vitro* *C. burnetii*-stimulated cytokine production assays (IFN-γ and Interleukin-2 (IL-2)) every 4 weeks and an 18F-fluorodeoxyglucose positron emission tomography combined with CT (FDG-PET/CT scan) before start of treatment and after 6 months. The *C. burnetii*-induced reactive oxygen species (ROS) production in monocytes was also monitored.

Results: IFN-γ therapy was well tolerated. During treatment, *C. burnetii*-induced ROS production in monocytes did not change. Neither did TNF-α, IL-6 or IL-10 production upon *in-vitro* stimulation of peripheral blood mononuclear cells (PBMCs) with *C. burnetii*. The IFN-γ/IL-2 ratio initially declined from a high ratio (1325) to a low ratio (12) in one month after start of treatment. However, after 2 months, the ratio rose again and remained stable. Serological titres increased from IgG phase I 1:2048 to 1:4096 after 3 months and FDG-PET/CT after 6 months of therapy revealed increased activity at the site of the existing lesions and an increase of the size of the psoas abscess. Therefore, it was concluded that IFN-γ therapy was ineffective. IFN-γ was stopped, and the patient started with doxycycline in addition to claritromycine.

Conclusions: We describe an adult chronic Q fever patient treated with recombinant IFN-γ in addition to antibiotic treatment. However, after 6 months we were unable to show any beneficial effect on serologic titres, cellular immunity monitored by IFN-γ and IL-2 production assays, and FDG-PET/CT results deteriorated. Consequently, the strategy of IFN-γ supplementation was abandoned.
An express test of fluorescence analysis was developed for detection antibody to Coxiella burnetii and Rickettsia prowazekii. A multiplex antigens was used for the test. Its corpuscular antigens were conjugated with quantum dots of different colors. Antigens of C. burnetii was conjugated with nanopatical CdSe green fluorescence (diameter 2.5nm, wavelength 513nm), R. prowazekii – with CdSe red fluorescence (diameter 4.8nm, wavelength 620 nm) and was combined in multiplex diagnosticum. Multiplex diagnosticum can to detect antibody to C. burnetii and R. prowazekii in one sample. Labeled C. burnetii form with specific antibodies agglutinates, fluorescent green color, and labeled R. prowazekii – agglutinates, fluorescent red color. In the absence of specific antibodies, isolated green corpuscles of C. burnetii and isolated red corpuscles of R. prowazekii were observed. 12 sera of patients with Brille-Zinsser disease, 7 sera of with patients Q-fever, 56 sera of febrile patients and 22 sera of health people was tested in nano-agglutination test and indirect immunofluorescence assay (IFA). Sensitivity for to R. prowazekii was 100%. Sensitivity for C. burnetii was detected 100%. Sera from 56 of febrile patients and 22 sera of health people were demonstrated negative results. Nano-agglutination is a sensitive, specific and simple test that, in contrary of IFA, needs 1 hour.
Q fever is a worldwide zoonosis caused by *Coxiella burnetii*, which can be acute or chronic. The diagnosis of Q fever relies mainly upon serology, the most commonly used method being the immunofluorescence assay, qPCR and immunochemistry (IHC) was also used but numerous cases have presented discrepancies between these two last methods. However, Fluorescence in situ hybridization (FISH) technique has been developed as a complementary tool. We analysed by qPCR, IHC and FISH, 43 different samples (valves, hepatic, splenic, vascular, bone and lung biopsy) from patients with confirmed Q fever from 2013 to 2016. QPCR has been used to detect *C. burnetii* DNA in clinical samples using primers derived from the htpAB-associated repetitive element. IHC and FISH were performed on paraffin-embedded sections, for IHC with the anti-*C. burnetii* mouse monoclonal antibodies and for FISH with specific 16S rRNA probe. Among 43 samples, 14 were positive for *C. burnetii* by FISH, 11 were positive by qPCR and 2 were positive by IHC. In 11 qPCR positive samples, 0 (0%) were positive by IHC and five (45%) were positive by FISH. In contrast, among 33 qPCR negative samples, six (18%) were positive by FISH and two (6%) were positive by IHC. Finally, seven were positive by FISH but negative by qPCR and IHC. The sensitivity of FISH was 70%, of qPCR assays was 55%, and of IHC was 10%. No significant difference was found between qPCR and FISH ($p=0.28$) or between IHC and FISH ($p=0.09$). We demonstrate that in our series samples, FISH was more sensitive than qPCR and IHC, and allowed to detected *C. burnetii* in cases of PCR and IHC negative. So, this technique can be used as a complementary tool for *C. burnetii* detection and may be included in the diagnostic strategy for Q fever.
In some rural areas of South Africa, where tick-borne diseases could be underestimated, humans are in close contact with donkeys commonly infested with ticks. During 2014, 379 ticks were collected from 74 donkeys in the Limpopo Province. Ticks were grouped in 100 pools (9 *Amblyomma hebraeum*, 1 *Hyalomma rufipes*, 3 *Hyalomma truncatum*, 8 *Rhipicephalus appendiculatus*, 2 *Rhipicephalus decoloratus*, 1 *Rhipicephalus simus*, 76 *Rhipicephalus evertsi*). The presence of *Rickettsia* (*ompA*/*ompB*), *Anaplasma/Ehrlichia* (*16S-rRNA/groEL*) and *Coxiella burnetii* (*IS1111/htpAB*) was analyzed by PCR. *Rickettsia africae* and *Rickettsia aeschlimannii* were amplified in 12 (6 *A. hebraeum*, 1 *R. appendiculatus*, 5 *R. evertsi*) and 4 pools (1 *H. rufipes*, 1 *R. appendiculatus*, 2 *R. evertsi*) respectively. The *R. simus* pool showed an infection with *Rickettsia* spp. The *ompA* and *ompB* sequences reached 97.9% and 99.0% of maximum identity, respectively, with those corresponding to *R. africae* as a validated *Rickettsia* species. *Anaplasma and/or Ehrlichia* species were amplified in 3 pools. Specifically, the *groEL* sequences demonstrated the presence of *Ehrlichia canis* in 2 *R. evertsi* pools. In one of them, the 16S-rRNA amplicon showed maximum identity with *Anaplasma bovis*. A 16S-rRNA fragment closest to *Anaplasma ovis* was obtained from one *A. hebraeum* pool. Moreover, the IS1111 sequence of one *A. hebraeum* pool showed homology with *C. burnetii*. In conclusion, a potential new *Rickettsia* species has been detected in *R. simus* from South Africa, although more genes should be analyzed. The presence of *R. africae*, *R. aeschlimannii*, *A. ovis*, *A. bovis*, *E. canis* and *C. burnetii* has been corroborated in this country.
Q fever is an emerging disease in French Guiana, a French overseas department located in the northeast of South America. In 1996, three patients were hospitalized in the capital Cayenne, resulting in one death. The incidence of the disease increased from 37 to 150 cases / 100,000 inhabitants between 1996 and 2005. From 2004 through 2007, the prevalence of *Coxiella burnetii* in community-acquired pneumonia was 24.4%, one of the higher proportions ever reported. The most common clinical presentation of the disease is a severe acute pneumonia, suggesting a contamination by the respiratory route, resulting from the inhalation of infectious aerosols. *C. burnetii* MST 17 was found as the unique clone causing Q fever in French Guiana, responsible for an exacerbated immune response and is to date the most virulent genotype of *C. burnetii*. The epidemiology of the disease has specific features as domestic ruminants are not usually infected. The search for a potential animal reservoir led us to carry on a survey on dogs. From 2013 through 2016, we sampled 261 dogs (pets and shelter dogs), including 202 females and 59 males, from Cayenne and Kourou, the two main cities of Guiana. Serology was performed using indirect immunofluorescence technique which allows the detection of antibodies (IgG and IgM) against phase I and II antigens characterizing the antigenic variation of *C. burnetii*. The antigens were produced from *C. burnetii* Nine Mile. The cutoff for phase I and II, was 1/50 for IgG and 1/25 for IgM. Real time PCR (qPCR) was performed on vaginal swabs targeting the repeated sequence IS1111. The DNA from the Nine Mile II strain was used as a positive control, and sterile water was used as a negative control. In 2014, we also carried out a qPCR on blood (total blood with EDTA anticoagulant) from 59 dogs. In serology, among 154 dogs, one was IgG phase I positive (1/400), IgG phase II positive (1/200), IgM phase I positive (1/100) and IgM phase 2 positive (1/50). Two other dogs were positive in IgG only (1/100 and 1/50). Globally the seroprevalence was 2% (3/154). Eighteen vaginal swabs among 202 were qPCR positive, including 6 with Ct<35. The sequencing performed on two samples showed 100% identity with *C. burnetii*, but a genotype different from MST 17. In the blood samples, two dogs from 59 were positive (Ct: 36). These results indicate that dogs in French Guiana are probably not responsible for the human epidemic of Q fever. The natural reservoir of *C. burnetii* MST 17 might be wildlife, such as the three-toed sloth, as we showed in another study.
The knowledge on rickettsioses in Senegal has increased in recent years by highlighting the significant incidence of human infections by *Rickettsia felis* in this region. The role of domestic animals in the epidemiological cycle of rickettsial infections transmitted by arthropods is not yet clear. We studied the evidence of rickettsial infection in 78 dogs and 48 cattle from the villages of Casamance, southwestern province of Senegal. In 2012, we collected blood and ticks (N = 142) from these 126 animals. *Rickettsia* genus-specific qPCR targeting the *gltA* gene was performed on the blood (EDTA) and indirect immunofluorescence was performed with *R. africae*, *R. conorii*, *R. massiliae* and *R. felis* antigens. All PCRs on the blood were negative. In contrast, 3/18 (16.7%) of *Amblyomma variegatum* ticks collected from cattle contained *Rickettsia africae* DNA. All 124 *Rhipicephalus* spp. ticks collected from dogs were negative in PCR for rickettsiae. Dog's sera were tested positive for *R. africae* (46%), *R. conorii* (31%), *R. massiliae* (24%) and *R. felis* (9%). Cattle sera reacted with antigens of *R. africae* (58%), *R. conorii* (42%) and *R. massiliae* (6%). These results show the involvement of domestic animals in the circulation of spotted fever group rickettsiae in Senegal. The infection do not appear to persist in animals that remain asymptomatic. Domestic animals, however, may serve the sentinels for monitoring of spotted fevers taking in consideration the high prevalence of such infections in Senegal.
Coxiella burnetii (C. burnetii) is an obligate intracellular pathogen and the etiologic agent of Q Fever in many animal species and humans. Infections in cattle are generally asymptomatic and the most frequently reported clinical sign is late abortion. Several studies reported the association between C. burnetii and post-partum metritis, stillbirth and weak offspring. However, no solid evidence supports the hypothesis of C. burnetii as infectious agent responsible for other reproductive disorders such as endometritis, subfertility and retained fetal membranes. For this study, microbiological, histopathological and PCR evaluation of 40 uterine biopsies obtained from dairy cattle with poor fertility were performed. Formalin-fixed, paraffin embedded uterine biopsies sections (4 μm thick) were stained with Haematoxylin and Eosin and Van Gieson. The uterine content was concurrently tested for microbiology assays. The endometrial biopsies and uterine content of thirty cows did not show any significant lesions and no pathogens were identified by bacteriological culture and PCR. Ten cows resulted PCR positive for C. burnetii, and negative for other pathogens by bacteriological culture and PCR. Histological examination of these cases revealed a mild to severe chronic inflammation admixed with deep necrosis, perivascular and periglandular fibrosis in the most severe cases. Immunohistochemical evaluation of C. burnetii-positive biopsies confirmed the presence of the microorganism within the cytoplasm of macrophages identifying, for the first time, the presence of C. burnetii in macrophages at the site of inflammation in bovine endometrium.
Coxiella burnetii a small Gram-negative intracellular bacterium is the causative agent of Q fever which is a zoonotic disease with a worldwide distribution. Domesticated ruminants represent the main reservoir of the disease but the bacterium is able to infect a wide range of hosts including humans, arthropods and invertebrates. Virulence studies of Coxiella strains usually require a suitable animal model. However, mammalian models are costly and associated with many ethical constraints. Recently, the alternative infection model using Galleria mellonella was used to study the virulence of several bacterial as well as fungal pathogens. Moreover, the G. mellonella larvae model has been used to identify virulence genes using NMII C. burnetii mutants. In our study we describe its use for the characterization of ruminants C. burnetii field strains.

First, the bacterial multiplication in the larvae was assessed by qPCR using the Nine Mile reference strain. The lethal effect of the bacteria on larvae was then assessed for several strains (n= 9), representing isolates from different ruminants species. To do this, serial dilutions for each strain were evaluated and the mortality was recorded for 96 hours. Finally the LD50 of each strain was calculated from the obtained data.

We observed that the multiplication of the bacteria occurred into the hemolymph of galleria larvae and the larval death occurred in a dose-dependent manner. Moreover, the model enabled a significant distinction between some of the C. burnetii examined strains. The G. mellonella constitutes a good alternative infection model for Coxiella, capable of distinguishing between the virulence properties of different strains. Moreover, it is cheap, easy to establish and to maintain, and does not require ethical approval. Further studies are needed to evaluate its use as a screening model for virulence differences of Coxiella burnetii strains as well as a viability model for the determination of the infectious risk in biological as well as environmental samples.
Q fever is a worldwide zoonosis caused by the gram-negative obligate intracellular bacterium *Coxiella burnetii*. Following the recent outbreaks in the Netherlands, a hyper virulent clone was found to cause severe human cases of Q fever. In livestock, Q fever clinical manifestations are mainly abortions. Although various factors may explain the bacterial dissemination or the abortion rates, *C. burnetii*’s virulence remains understudied, especially in enzootic areas. Thus *C. burnetii*’s strain characterization is of high importance to evaluate the level of infectious risk, especially in enzootic areas.

In this study, the infectious potential of three *C. burnetii* isolates collected from French small ruminants farms were compared to the reference strain Nine Mile (in phase II and in an intermediate phase) using an *in vivo* (CD1 mice) model. Mice were footpad inoculated with $10^5$ live bacteria previously categorized by propidium monoazide-qPCR targeting the *icd*-gene. The spleen and popliteal lymph node were harvested 10 days post-inoculation (p.i) and the strain invasiveness was assessed by qPCR assays targeting the *icd*-gene.

The results showed that avirulent (phase II) strain failed to pass the popliteal barrier and thus were unable to colonize the spleen whereas colonization of both the spleen and popliteal lymph node was observed with virulent strains. In view of these results, further *C. burnetii* isolates from French farms will be studied using the above-mentioned *in vivo* model as well as an *in vitro* cell culture model based on ovine and bovine macrophage cells.

The implemented mouse model allowed a significant differentiation between strains’ invasiveness on biological host and therefore identifying distinct virulence profiles. Moreover, we believe that our model enables not only the distinction between wildtype *C. burnetii* strains’ pathogenicity but might be useful as well to test genetically modified *C. burnetii* to identify virulence markers.
Q fever is an important disease of relevance for veterinary and public health sectors. Vaccination protocols are applied in humans in specific contexts, namely in high risk persons. In animals, vaccination is used as control measure to limit bacterial spreading between livestock herds and decrease the risk of human infection. In this work, the efficacy of livestock-derived whole-cell vaccines (caprine CbBEC1 and bovine CbBEB1) were compared upon infection with homologous and heterologous livestock (CbBEC1 and CbBEB1) challenge and to a genetically distant heterologous challenge with a tick-derived isolate in the BALB/c model. High production of IgG1 (Th2-type response) was elicited by the two vaccines. Levels of IgG2a (Th1-type response) however depended on the administrated vaccine, with the CbBEB1 vaccine eliciting the response with the highest magnitude. Upon challenge, CbBEC1 and CbBEB1 vaccine protected efficiently against livestock but lesser to the genetically distant isolate challenge. This was observed by effects on spleen weight, decreased bacterial load in spleen and lungs and less histopathological lesions in spleen. This work identified levels of IgG2a (post-vaccination and post-challenge), total IgG post-vaccination and the measure of spleens’ weights post-challenge as indicators to evaluate vaccine efficacy against C. burnetii in the mouse model. Taken together, these findings highlight the importance of the Th1-type response in controlling C. burnetii infection but also suggest the presence of host species-specific factors to modulate this response.
Q fever is a zoonosis caused by *Coxiella burnetii* (*Cb*). Previous studies hypothesized an epidemiological role of rodents in the spread of Q fever infection among ruminants. However, few data are available to support this hypothesis. During 2016, 127 brown rats were captured in three Italian dairy cattle farms. Molecular diagnosis for *Cb* was performed on 127 rat spleens and 3 bovine tank milk samples collected from the same farms. A real-time PCR revealed positivity in 11 rat spleens and in all milk samples. Nine rats' DNAs and 2 bovine milk samples were selected for molecular characterization by means of SNP analyses and MLVA genotyping. In order to improve strain characterization, the MLVA panel included two more loci (MS21 and MS36) as of the original Dutch 6-locus panel. For some samples, only partial MLVA genotypes were obtained because of the high ct values (>34). SNP typing on 10 markers showed the presence of a unique genotype, the SNP-2, for both rats and cattle. MLVA on rat's strains showed the same profile in 5 loci, as observed in cattle's strains, and variable repeats for MS23, MS24 and MS34. *Cb* strains collected in this study from rodents and cattle are more related to each other, with respect to other ruminant's strains as presented in available databases, confirming rat susceptibility to the infection caused by *Cb* cattle's strains in natural conditions. None species-specific genotype in rats was observed. It remains to elucidate their role in transmission and possible link with the sylvatic cycle.
Coxiella burnetii is the causative agent of human Q fever, which presents as acute or chronic disease, with symptoms ranging from fever and fatigue to fatal endocarditis. C. burnetii is an intracellular bacterium that replicates within an acidic, lysosome-like parasitophorous vacuole (PV) in human alveolar macrophages. During intracellular growth, C. burnetii uses a Type IV Secretion System to deliver bacterial effector proteins into the host cytoplasm. Multiple effectors localize to and/or disrupt the endoplasmic reticulum (ER), but their role in infection is unknown. During microbial infection, unfolded nascent proteins may exceed the folding capacity of the ER, activating the unfolded protein response (UPR) to revert the ER to its normal physiological state. Some intracellular pathogens manipulate the UPR and/or ER-Associated Degradation (ERAD) pathway to promote survival in host cells. Here, we investigated the impact of C. burnetii on activation of the three arms of the UPR. The PERK arm was activated during C. burnetii infection of human macrophages, leading to increased levels of phosphorylated eIF2α and nuclear translocation of the transcription factor ATF4. ATF4 normally drives expression of the pro-apoptotic protein CHOP; however, our results suggest C. burnetii prevents expression and downstream effects of CHOP, such as apoptosis. Importantly, UPR and ERAD inhibitors antagonize C. burnetii growth in macrophages, indicating these processes are needed for efficient infection. Collectively, our data indicate C. burnetii modulation of the UPR via distinct arms is required for intramacrophage growth.
Human Q fever is caused by the intracellular pathogen, *Coxiella burnetii*. Q fever can present with acute flu-like symptoms or progress to chronic, severe endocarditis. After human inhalation, *C. burnetii* travels to the alveolar spaces and is engulfed by macrophages. The pathogen transits through the phagolysosomal maturation pathway and resists acidic pH upon fusion with lysosomes to form a parasitophorous vacuole (PV) for replication. Previous studies showed that *C. burnetii* replicates efficiently in primary alveolar macrophages in ex vivo human lung tissue. Although *C. burnetii* replicates in most cell types in vitro, the pathogen does not grow in alveolar epithelial cells in human lung tissue. Here, we assessed whether *C. burnetii* replicates in pulmonary epithelial cells apart from the lung environment. We found that *C. burnetii* infected, formed a PV, and replicated in airway, but not alveolar, human immortalized epithelial cell lines. However, growth curve analysis showed that *C. burnetii* replication was delayed in airway cells compared to macrophages. Additionally, the cytokine profiles of infected epithelial cells were compared to macrophages to define cell-specific innate responses. Our results suggest alveolar epithelial cells do not support *C. burnetii* growth outside the lung environment, providing a new cell-specific context for defining anti-*C. burnetii* activity. Additionally, airway epithelial cells may represent an uncharacterized replication niche for the pathogen. Collectively, this study defined parameters of infection for future use in characterizing *C. burnetii* interactions with primary human epithelial cells.
Follow-up of intra-herd circulation of *Anaplasma phagocytophilum* strains in cattle farms in France and Germany by molecular typing

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*Anaplasma phagocytophilum* is a zoonotic tick-borne intragranulocytic alpha-proteobacterium. It infects a large range of hosts, including wild and domestic ruminants, dogs, horses, rodents and humans. *A. phagocytophilum* is the causative agent of tick-borne fever in domestic ruminants, which has significant economic impact in farms in Europe. Molecular epidemiology represents a powerful approach to elucidate the complex epidemiological cycles of multi-host pathogens, such as *A. phagocytophilum*. However, there is little information about the strains circulating within a defined bovine environment. Our objective was to assess the presence and genetic diversity of *A. phagocytophilum* obtained from the same farms over time. We performed blood sampling repeatedly on cattle from two neighbor farms in Sologne, a French wet region known to be a suitable environment for ticks' development. Regularly in these farms, several animals presented clinical signs strongly suggestive of tick-borne diseases, of which some of them died. In addition, we had access to DNA from cows from North-Rhine-Westphalia in Germany. Blood samples were also collected in these animals over time. The presence of *A. phagocytophilum* DNA was detected by *msp2* qPCR on 20 cows in French farms and 6 cows in German farms. Some animals (5 from France and the 6 German cows) were still positive two months or more after the collection of the first sample, and even after 17 months for one of them. The positive samples were submitted to molecular typing using seven genetic markers (*msp4*, *typA*, *pleD*, *ctrA*, *polA*, *recG* and *GroEL*). The results showed that at least two strains (sharing the same profiles in both countries) circulate within farms, both in France and in Germany, with some animal presenting co-infections, only found in France. Concerning the paired samples obtained from the same animals at two different periods, the profiles obtained over time were not identical. Our results suggest the absence of a long-lasting protective immunity and that, according to the *A. phagocytophilum* profiles observed, either reinfection of a given cow by a different strain circulating concomitantly in the herd or prolonged infection by the same strain that evolved with time, occured.
Anaplasma phagocytophilum is an obligate intracellular gram-negative bacterium, is the causative agent of granulocytic anaplasmosis, condition that affects several species of wild and domestic mammals, including horses. It is a tick-borne disease transmitted especially from *Ixodes* ticks. *A. phagocytophilum* preferentially infects granulocytic leukocytes, primarily neutrophils and eosinophils. In Chile *A. phagocytophilum* has not been previously reported by molecular methods. The aim of this study was to perform molecular detection of *A. phagocytophilum* in horses of this particular racecourse in Chile. One hundred and sixty nine horses were randomly selected and sampled from the entire population of horses in this racecourse. Quantitative Real Time PCR (qPCR) assays to amplify *msp2* gene from *A. phagocytophilum* were performed. A Beta-actin protein (*ACTB*) was used as endogenous gene. A synthetic sequence (gBlocks® Gene Fragments) constructed from *msp2* gene sequences of the *A. Phagocytophilum* and ultra pure nuclease free water were used as positive and negative controls, respectively. All samples were positive for *ACTB* gene. The melting temperature of the amplified products has an average value of 80.01 °C similar to the positive control. The occurrence of *A. phagocytophilum* was 10.05% (17/169). This is the first molecular description of *Anaplasma phagocytophilum* in Chile and one of the few in south America.
Anaplasma phagocytophilum is an obligate intracellular gram-negative bacterium, that parasites leukocytes and endothelial cells of mammals. The disease is reported mainly in tropical and sub-tropical regions and has zoonotic significance. Some ticks involved in the transmission, as Amblyomma spp. and Rhipicephalus sanguineus sensu lato, are widely distributed in Paraguay, however; A. phagocytophilum has not been previously reported in the country. The aim of this study was to determine the molecular prevalence of A. phagocytophilum in domestic dogs from Paraguay. Three hundred and eighty four domestic dogs from six districts of Asunción city were sampled. Quantitative Real Time PCR (qPCR) assays to amplify msp2 gene from A. phagocytophilum were performed. RPS19 was used as endogenous gene. All samples were positive for RPS19 gene and melting temperature of the amplified products was 80.00 ± 0.72°C. The prevalence of A. phagocytophilum was; 6.51% (25/384) being similar to that described in dogs from Brazil (7.11%), wich is the only country in South America where the agent was detected before in dogs. This is the first description of A. phagocytophilum in Paraguay.
The relevant research funding made during the last decade has further elucidated the nature of the syndrome caused by anaplasmosis and ehrlichiosis in the infected host, the importance of animals as a reservoir of this bacteria, and the potential zoonotic of some species. In Africa, the impact of Rickettsiales bacteria on animals remains a neglected area of research. In addition, potential vectors of ticks, for most of the species identified in Africa, remains misunderstood. In Senegal, few data are available about the epidemiology of Anaplasmataceae species that infect animals and ticks. The aim of this study was to review and assess the prevalence of Anaplasmataceae species infecting and currently circulating in and between cattle, sheep, goats, horses, dogs and ticks in three areas of Senegal near Keur Momar Sarr (Northern region), Dielmo and Diop in the Sine Saloum (Centrale region), and in Casamance (Southern region). In addition, the aim of this study was to better understand the molecular characterization of these Anaplasmataceae and to study their phylogenetic position. Two hundred and four ticks and 433 blood samples were collected from ruminants, horses, donkeys and dogs. The overall prevalence of Anaplasmataceae infection in the sampled animals was 41.1%, whereas in ticks only 0.9% of collected ticks were found positive. We have identified the following pathogenic bacteria as infecting both animals and ticks: Anaplasma ovis, A. marginale, A. centrale, A. platys and Ehrlichia canis. We have also discovered a potentially new species of Anaplasma cf. platys and another provisionally named here as Candidatus Anaplasma africæ. However, except for E. canis, none of the other species were identified in ticks. These findings suggest that sub-Saharan African animals are one among other important reservoirs of Anaplasmataceae species. Nonetheless, other studies are needed to describe the complete epidemiological ticks-host-Anaplasmataceae network implicated in the transmission of Anaplasmataceae species in this region of the world.
Candidatus Neoehrlichia mikurensis (CNM) is an emerging tick-borne pathogen. It primarily infects immunocompromised dogs and humans, though in China symptomatic immunocompetent human patients were described. Ticks of the genus *Ixodes* likely serve as vectors, whereas rodents serve as CNM hosts. Most genetic studies have focused on diversity of 16S rRNA gene and *groEL* operon. Analysis of nucleotide sequences of these two genes in Europe revealed no genetic diversity, while comparison of the two genes of CNM strains obtained from ticks and rodents in Europe and Asia revealed marked geographic clustering, suggesting the existence of a European and Asian CNM genetic cluster. Some other genes have also been analysed, but only *lipA* and *clpB* showed some diversity. Slovenia represents a gap in the reports on the presence of CNM in the Central Europe. Therefore, we screened samples obtained from ticks *I. ricinus*, different species of rodents, dogs and humans for the presence of a smaller part of the *groEL* operon of CNM according to published literature. All samples from dogs and human patients tested negative while several tick and rodent samples were positive. In positive samples sequencing of the larger part of *groEL* operon and 16S rRNA gene was performed. In addition, selected samples were further tested for genes *clpB* and *lipA*. The results of our preliminary study indicate that ticks *I. ricinus* and different species of rodents in Slovenia harbour CNM. Sequences of Slovenian CNM phylogenetically cluster within the European lineage of CNM and no genetic diversity of 16S rRNA gene and *groEL* operon was observed.
Argasid ticks (soft ticks) are blood-feeding arthropods that may parasitize rodents, birds, humans, livestock and companion animals. Ticks of the *Ornithodoros* genus are known as vectors of relapsing fever borreliae in humans. In Algeria, relapsing fever borreliae and other bacterial pathogens transmitted by Argasid ticks are poorly known. Between May 2013 to October 2015, we investigated the presence of soft ticks in 20 rodent burrows, 10 nests of the yellow-legged gull (*Larus michahellis*) and several animal shelters were prospected in 5 sites located in two different bioclimatic zones of Algeria. Six species of Argasid ticks were identified morphologically and by 16S rRNA gene sequencing. The prevalence of *Borrelia* spp., *Bartonella* spp., *Rickettsia* spp. and Anaplasmataceae bacteria carriage was assessed by qPCR template assays in each specimen. All samples identified as positive by qPCR were confirmed by a standard PCR followed by sequencing the amplified fragments. Two *Borrelia* species were identified: *Borrelia hispanica* in *Ornithodoros occidentalis* in Mostaganem, and a novel relapsing fever *Borrelia* in *Carios capensis* in Algiers. One new *Bartonella* genotype and one new *Anaplasmataceae* bacteria genotype were also identified in *Argas persicus*. The present study highlights the circulation of two *Borrelia* species known as agents of relapsing fever although this disease is rarely diagnosed in Algeria. Two other bacteria of unknown pathogenicity have been detected in Argasid ticks biting humans.
Human Granulocytic Anaplasmosis (HGA) is a zoonotic disease caused by *Anaplasma phagocytophilum* and transmitted by infected ticks. Clinical manifestations of HGA are similar to some other tick-borne infections (eg *Borrelia burgdorferi*) with flu-like symptoms, including fever, myalgia, rigors and headaches; rashes are however rare. HGA can be a serious and fatal illness (0.6% case fatality rate) presenting with breathing difficulties, haemorrhage, renal failure and neurological problems.

In the UK, 757 Anaplasma tests were performed in 2016 at the Rare and Imported Pathogens Laboratory (RIPL), primarily as a differential diagnosis in a subset of *Borrelia burgdorferi* investigations. HGA in RIPL is currently diagnosed using the Focus IgG indirect immunofluorescent antibody (IFA) assay, which utilises Anaplasma-infected HL60 cells. IFA-based assays are subjective and require experienced operators to interpret. As part of our service improvement commitment, we have performed a preliminary evaluation of the commercial Blot-Line Anaplasma IgM and IgG assays (TestLine Clinical Diagnostics), which are based on three purified antigens, as a potential IFA replacement. PCR is not currently available as a routine diagnostic test in the UK. We have carried out preliminary evaluation studies for two published PCR assays for Anaplasma. We will present our findings on the preliminary evaluations of both the IFA and PCR assays and our plans for future diagnosis of HGA in the UK.
P2-33) Denys Pogoryelov – *Chlamydia* energy metabolism revisited: V/A-ATPase in focus as a novel drug target

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*Chlamydia* species are the obligate intracellular parasites for which improper treatment develops in chronic infections and associated with multiple diseases. Chlamydial cells largely depend on the uptake of ATP from their hosts. However, the path of autonomous supply of ATP in the chlamydial cells is uncertain at the circumstances when ATP scavenging from the host cells is impeded.

The omics analysis indicate that chlamydial cells produce several bioenergetics protein complexes that may be involved in the regeneration of ATP autonomously by chlamydial cells via rudimentary respiratory chain. However, this hypothesis, up to now, lacks experimental verification. We aim to revise the processes of energy metabolism in *Chlamydia* cells, particularly by studying the functional role of putative Na⁺ transporting respiratory chain complexes and associated with it components towards the deeper understanding of chlamydial energetics and assessment of these components as novel targets for anti-chlamydial therapy in humans. We set to address chlamydial V/A-type ATPase as the putative drug target. Since no specific and medically relevant inhibitors of this type Na⁺/H⁺-V/A-ATPase are currently available, for validation of this target, we are in the process of designing new effective compounds by chemical ligand libraries screening and structure-based in silico docking pipelines.

Our current progress on the functional and structural characterisation of the chlamydial V/A-ATPase will be presented at the meeting.
Vitamin B9 (folate) is vital for cell growth and development in all organisms. Since ticks feed on folate-poor blood as their only food source and are incapable of de novo folate synthesis, the vitamin must therefore be obtained through symbiotic mutualisms. Previous research in our lab have shown that *Rickettsia* species phylotype G021, a bacterial endosymbiont that is maintained transovarially and transstadially in *Ixodes pacificus*, contains all genes (*folA, folC, folE, folKP, and ptpS*) of de novo biosynthesis of folate. However, relatively little is known about how the nutritional demand of vitamin B9 is met in *I. pacificus*. Gene expression by phylotype G021 at different stages of tick life cycle may be essential for nutrition and development of ticks. Here, different stages (eggs, flat larvae, engorged larvae, flat adults, and engorged adults) of *I. pacificus* were collected by feeding on New Zealand White rabbits. Gene expression of the genes of the folate biosynthetic pathway in different stages of ticks was measured by quantitative reverse transcription PCR. We detected that all five genes of the folate biosynthetic pathways of phylotype G021 are transcribed in the stages of the tick life cycle, including the developing eggs. In addition, the transcription profile of the *folA*, *folC*, and *ptpS* genes in the engorged larvae showed an increase of mRNA expression of 5.4 fold, 1.8 fold, and 2.2 fold, respectively, compared with the mRNA expression of the flat larvae. Furthermore, Recombinant FolA and FolE proteins were overexpressed and purified by affinity chromatography. SDS-PAGE results showed that both recombinant rickettsial proteins were overexpressed in BL21(DE3) *E. coli*. The specific activity of purified FolA (dihydrofolate reductase) and FolE protein (GTP cyclohydrolase I) of phylotype G021, detected by in vitro enzyme assays, was calculated to be 16.1 U/mg and 7.9 U/mg, respectively. These data indicated that phylotype G021 contributes to, at least partially, nutrition and development of *I. pacificus*. 
Chantal Bleeker-Rovers – Outcome and complications of Q fever, lessons from the Dutch national database

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Q fever is a zoonosis caused by the intracellular Gram-negative coccobacillus *Coxiella burnetii*. Following primary infection, 1-5% of all patients develop chronic Q fever with endocarditis, infected aneurysms or infected vascular prostheses as most important manifestations. The duration between primary infection and manifestation of chronic infection may be several years and a lot is still unknown about pathogenesis, optimal diagnosis, choice of treatment, and treatment duration. Between 2007 and 2010, there was a large Q fever outbreak in the Netherlands. It is estimated that over 40,000 humans were infected with major impact on physical and psychological health. Following this outbreak, all known chronic Q fever patients were included in an ongoing nationwide registration, the Dutch National Chronic Q fever Database, now including 249 proven chronic Q fever patients and 74 probable chronic Q fever patients. This large cohort provides the unique opportunity to assess outcome of diagnostic procedures, occurrence of complications, mortality rate, and choice and duration of treatment in an otherwise rare disease. Results from this database on the value of FDG-PET/CT in localizing infectious foci and timely diagnosis of complications, alternative treatment regimens to doxycycline and hydroxychloroquine, and the importance of determining doxycycline levels on outcome will be discussed.
Recent advances in the clinical investigation of *Coxiella burnetii* infections have led to major changes in the understanding of clinical forms of Q fever. Until recently, two main forms have been described: acute and chronic Q fever. Thus, the patients were distinguished by the duration of the symptoms (more or less than 3 months) without distinction of the infectious localizations. However, systematic screening for valvulopathy, the advent of F18 FDG PET scan, the discovery of the major role of anticardiolipin IgG, and the demonstration of a causal link between *Coxiella burnetii* infection and lymphoma led to withdraw this dichotomy. Indeed, the discovery of vegetations from the first week of symptoms in patients with a serological primo-infection led to the description of acute Q fever endocarditis. The discovery of the role of anti-phospholipid antibodies led to the identification of patients at risk of manifestations of the antiphospholipid antibody syndrome (particularly acquired valvulopathy including vegetation and thrombosis). These complications are very early and follow the secretion of antiphospholipid antibodies which is explosive and maximal at the beginning of the primary infection before a gradual decrease. The F18 FDG PET scan allowed the diagnosis of lymphadenitis specifically associated with the development of lymphoma. The identification of these new clinical forms and the abandonment of the “chronic Q fever” term will improve the management and the prognosis of patients with Q fever by an earlier and more specific management.
Chronic infection with Coxiella burnetii (chronic Q fever) can cause life threatening conditions such as endocarditis, infected vascular prostheses and infected arterial aneurysms. Prognosis, nature of complications and their risk factors are largely unknown. We performed a retrospective cohort study to evaluate mortality and complications (assessed by predefined criteria) in chronic Q fever patients registered in the Dutch national chronic Q fever database. We identified 439 chronic Q fever patients (73% male, mean age 65 years). Complications occurred in 166 patients (38%): 153/249 with proven (61%), 11/74 with probable (15%) and 2/116 with possible chronic Q fever (2%). Most complications occurred prior to initiation of therapy (n=101; 61%). Most commonly observed complications were acute aneurysms (n=63; 14%), heart failure (n=55; 13%) and non-cardiac abscesses (n=45; 10%). Factors associated with complications were positive serum PCR at any moment during disease (OR2.25; 95%CI 1.36-3.72) and presence of prosthetic material prior to diagnosis (OR1.79; 95%CI 1.07-2.99). Overall mortality was 27% (n=118). Mortality was considered related to chronic Q fever in 66 patients (15%): 63 patients (25%) with proven, 3 patients (4%) with probable and no patients with possible chronic Q fever. Complications predicted chronic Q fever-related mortality (OR8.20; 95%CI 3.65-18.45). We conclude that complications occur frequently, and that they strongly contribute to mortality in chronic Q fever patients. Patients with proven chronic Q fever have the highest risk of complications and chronic Q fever-related mortality. Prognosis in patients with possible chronic Q fever is favorable in terms of complications and mortality.
Donato Antonio Raele – *Coxiella burnetii* and *Borrelia burgdorferi* sensu lato in poultry red-mites, *Dermanyssus gallinae* related to red-mite dermatitis outbreaks in city-dwellers, in Italy

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The poultry-red-mite (PRM), *Dermanyssus gallinae* is a hematophagous nest-dwelling parasite of birds; occasionally, it bites humans, inducing dermatitis. PRM-attacks in residential settings in association with synanthropic birds are increasing; the possibility that the mite may be involved in the transmission of pathogens, represents an additional concern to the parasite. Aim of this study was investigated PRMs from human dwellings for presence of zoonotic pathogens. A total of 88 PRMs from pigeon/sparrow nests, and related to 10 outbreaks of PRM-dermatitis occurred in city-dwellers in 2001-2016 years, were pooled (11 pools), crushed and their DNA extracted. Five different PCR assays were performed using specific primers for detection of *Coxiella (C.)* spp (16S-gene), *Chlamydophila* spp (16S-gene), *Rickettsia* spp (17KDa-gene), *Borrelia (B.)* burgdorferi sensu lato (groEL-gene) and *Bartonella* spp (ITS-gene). PCR products were sequenced and the obtained sequences compared with ones present in GenBank using BLAST. Three pools from three PRM-outbreaks resulted positive, of which one to *Coxiella* spp and two to *B. burgdorferi* s.l.. The sequenced amplicons showed 100% of identity with 16S gene of *C. burnetii* and 99% of identity with the corresponding sequences of *B. afzelii*. For the first time, we report the presence of *B. burgdorferi* s.l. in PRMs; the detection of *C. burnetii* also confirms data from of other studies. Birds, mainly pigeons are well known harbouring both these agents; their nests are highly infested by *D. gallinae* and mite-bird transmission has been also demonstrated for *C. burnetii*. However, further studies are needed to understand the role of PRMs in the epidemiology/ transmission of these pathogens and the human health related risk.
Raquel Alvarez-Alonso – Progression of *Coxiella burnetii* contamination in the air and environment in naturally infected sheep flocks during two consecutive lambing seasons

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*Coxiella burnetii*, the causative agent of Q fever, causes abortion in small ruminants and infected animals shed the bacteria at lambing contaminating the farm environment, where it can survive during long periods. Contaminated aerosols are the main source for human infection. To study the evolution of *Coxiella burnetii* infection in four naturally-infected sheep flocks where no specific control measures had been implemented, vaginal swabs, milk and faeces from a maximum of 40 ewes and 40 yearlings per flock as well as dust samples were taken within one week after lambing during two consecutive lambing seasons (2015/16 and 2016/17). Aerosols were also collected outdoors and indoors after lambing at monthly intervals. Analysis of all samples by Real-Time PCR targeting the IS1111 gen showed different patterns of shedding, indicating that Q fever was introduced in each flock at different times. During the first lambing season, an average of 66.3% ewes (120/181) and 60.5% yearlings (69/115) shed *Coxiella* through at least one of the excretion routes. The following lambing season a significant decrease in ewes shedding was observed (10.0%, 16/160). Independently of the percentage of shedders, all farms presented positive aerosols. Most of the dust samples were *C. burnetii*-positive but a progressive decrease in bacterial load was observed. Significant correlations between percentage of animal shedders and bacterial burden in dust and indoors aerosols were found. Studies on *C. burnetii* viability in environmental samples are in progress to determine how long *C. burnetii* remains viable in farms with a previous episode of Q fever.

**Acknowledgements:** Funded by INIA RTA2013-00051-C02-01
Coxiella burnetii can cause Q fever in infected humans and animals. Although infected pregnant small ruminants are the main risk for human Q fever, not much is known about the dynamics and clinical outcomes of C. burnetii infections in small ruminants before pregnancy. Therefore we studied the effect of C. burnetii challenged goats before pregnancy and successive breeding. Fifteen nulliparous goats were intranasally challenged with the Dutch outbreak strain 3262. At 30 weeks of age, goats were naturally bred after oestrus induction and synchronisation. Ten animals became pregnant. Four control goats followed the same regime, except for the inoculation and were pregnant as well. To assess the infection status, serum samples for ELISA were taken weekly till 7 weeks after inoculation. To assess the excretion of C. burnetii, vaginal swabs were taken 2 and 4 weeks post breeding and placentas were inspected and sampled at parturition. Samples were tested by single copy C. burnetii PCR. Two weeks post inoculation, antibodies started to rise, indicating a successful inoculation. Infected goats and control goats showed no significant difference in gestation, no abortion was observed. The placentas showed no abnormalities and no DNA of C. burnetii was detected in vaginal swabs or placentas. The results of this experimental infection show that pre-breeding infection with C. burnetii does not result in abortion nor in excretion of C. burnetii. Non-pregnant goats are probably effective in eliminating the infection so no bacteria are present that can infect the trophoblasts in the following pregnancy.
Monika Szymanska-Czerwinska – *Coxiella burnetii*-epidemiological situation with particular emphasis on genotypes circulating in population of Polish ruminants

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The aims of the research were to estimate the prevalence of *Coxiella burnetii* among dairy cattle herds and small ruminants in Poland and to determine the genotypes of positive samples using MLVA an MST methods.

A total of 515 milk samples from 173 herds were collected between 2014 and 2016. A total of 317 samples from 160 goat herds and 11 flocks of sheep were obtained for molecular analysis. 197 milk samples (155 BTM, 42 individual), 95 swabs, 8 tissue sections from stillbirth kids, 3 feaces and 14 placenta samples were tested. 61 of tested cattle herds (35.26 %) and 12.7% samples from small ruminants were positive in real-time PCR detecting the IS1111 element. Coxiella burnetii’s DNA was detected in 2 of 11 tested flocks of sheep and in 23 of 160 goat herds. MLVA-6 method was used to genotype 33 cattle milk samples and 12 samples from small ruminants with low Ct value, each from different herd. Three complete genotypes were obtained from 16 tested cattle samples and in four samples genotypes were almost complete. The MLVA genotypes differ in one of six loci and can be the microvariants of one genotype. According to available database all of them were found before, mainly in samples collected from cattle in Spain, France, Saudi Arabia, Netherlands, Switzerland, Hungary and also in human samples from France. Multispacer sequence typing revealed that all of genotyped samples both from cattle and small ruminants belong to ST20 genotype which frequently occurs in European and North-American cattle.

Molecular characterization of *Coxiella burnetii* strains circulating in Poland was crucial due to lack of current data. The last study was very limited and conducted ten years ago. Moreover, determination of the prevalence of this bacteria and characterization of its genotypes was essential to assess the potential risk for humans.

This study revealed that strains founded in Poland are commonly observe in many countries but, fortunately, they rarely caused the disease in humans.

This work was supported by the National Science Centre, Poland (Grant No. 2015/17/D/NZ7/00816).
Idir Bitam - Update of vector-borne Rickettsioses in the North Africa

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The present work consists of presenting an updated inventory of Rickettsiae detected and isolated in arthropod vectors in the Maghreb. Several new genotypes and new relationships between Rickettsia - Animals and Rickettsia - Arthropods have been identified and their isolations are underway which will allow to propose new names to these Rickettsiae. These bacteria are very frequent in this geographical situation and cause diseases including mortalities due to complications. This updating in the Maghreb will make it possible to propose a protocol of surveillance and prevention which will reduce the cases of mortality and morbidity in this region.
Various spotted fever group (SFG) Rickettsioses distribute worldwide, and show unique endemic characteristics by their causative agents, vectors and other factors. One side of worldwide diversity, many types of SFG Rickettsioses have been reported in Japan too. Recently, the genome information of many Rickettsiae is registered due to the introduction of the next-generation sequencing. Here, I show our experiences of genome analysis for Rickettsiae.

In 2011, we successfully isolated a novel SFG Rickettsia (SFGR) from a Japanese traveler returning from India. Addition to partial sequencing, we performed whole-genome analysis. According to the international genetic criteria using the nucleotide similarity for the \( rrs, \) \( gltA, \) \( ompA, \) \( ompB \) and \( sca4 \), and the results of whole-genome analysis, we proposed the new species, \( \text{Candidatus} \) Rickettsia indica.

The patients of Japanese spotted fever by \( R. \) \( japonica \) continue to increase. \( R. \) \( japonica \) is detected and/or isolated from many tick species. The life cycle of SFGR is thought to be closely associated with that of the tick. To clarify unique features and polymorphism of SFGR, we performed a nationwide, high-resolution phylogenetic analysis of \( R. \) \( japonica \). The results of genome comparison among isolates from various sources over the past 30 years demonstrated an extremely low level of genomic diversity. Our data provide novel insights on the biology and genome evolution of \( R. \) \( japonica \).

As our experiences, the whole-genome analysis of Rickettsiae might lead possibility of research on Rickettsiae.
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Scrub typhus caused by *Orientia tsutsugamushi* is the commonest rickettsioses and an important treatable cause of acute undifferentiated febrile illness (AUFI) in India. The eschar, a vasculitic reaction at the chigger bite site, is diagnostic but not always observed. In its absence, serological assays and molecular techniques become essential for confirming the aetiology. Moreover, the relation of eschar with disease severity is not well elucidated. The aim of this prospective study was to evaluate the relationship of eschar with severity of scrub typhus. Totally clinical details of 725 patients were recorded, whose clotted blood samples were received for scrub typhus IgM ELISA, over a six month period (July-December 2016). Of these 174 fulfilled the study criteria (fever duration ≤15 days, malaria smear and blood culture negative) at present 90 were evaluated by real-time PCR (qPCR). The qPCRs performed were for detection of 47 kDa antigen and RNAse P, using DNA extracted from the blood clots, in addition to the scrub typhus IgM ELISA. The data generated was analysed using Stata 13.1. Amongst the 90 individuals evaluated, RNAse P qPCR was positive in all, confirming good quality DNA had been extracted. Scrub typhus was diagnosed in 32, of whom one died and 17 had eschar. Poor agreement was observed between IgM ELISA and PCR for scrub typhus diagnosis, despite the former being positive in 27 patients and the latter in 15. IgM ELISA was most likely to be positive after 8 days (p=0.001), while PCR positivity diminished after 6 days. Individuals without eschar were more likely to have multi-organ dysfunction (MODS, ≥3 organ systems affected) than those with eschar (p=0.001). This preliminary study demonstrates that serological and molecular assays maximise the diagnostic yield. The 47 kDa qPCR is most useful for diagnosis in the first week of illness whereas from the second week onwards it is the IgM ELISA. Significantly, absence of eschar is associated with severe scrub typhus due to MODS. More samples need to be tested to confirm the validity of these findings. The remaining samples will be tested and the complete data will be presented at the meeting.
Ticks are currently considered as the second most important vector of human and animal diseases after mosquitoes. Consequently, the identification of ticks and associated pathogens is an important step in the management of these vectors. In recent years, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has been reported as a promising method for arthropod identification. Previous studies have shown that MALDI-TOF MS analyses of fresh or frozen tick legs may allow accurate tick species identification by analyzing protein profiles obtained by MALDI-TOF MS. A more recent study suggested that it was possible to identify ticks preserved in alcohol by MALDI-TOF MS. The aim of the present study was to improve tick leg sample preparation conditions for their identification by MALDI-TOF MS from Malian ethanol-preserved specimens collected in the field. In addition, the detection of microorganisms was done by molecular biology on tick half-bodies. A total of 1,333 ticks were collected from mammals in three distinct sites from Mali. Morphological identification allowed classification of ticks into 6 species. Among those, 471 ticks were randomly selected for molecular and proteomic analyses. Tick legs submitted to MALDI-TOF MS revealed a concordant morpho/molecular identification of 99.4%. The inclusion in our MALDI-TOF MS arthropod database of MS reference spectra from ethanol-preserved tick leg specimens was required to obtain reliable identification. When tested by molecular tools, 58.3%, 28.0%, 24.1% and 15.8% of the specimens tested were positive for *Rickettsia* spp., *Coxiella burnetii*, *Borrelia* spp. and *Anaplasma*aceae, respectively. These results support the use of MALDI-TOF MS in entomology, and improve the knowledge of tick species-diversity and tick-borne pathogens circulating in Mali.
Ida Chung – Evaluation of blood collection tube additives for *Rickettsia rickettsii*
conservation and detection

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*Rickettsia* PCR detection has been reported in clinical samples since the 1980’s, however literature does not identify a standard blood collection tube (BCT) additive for *Rickettsia* diagnosis. EDTA BCTs are widely reported, yet this anticoagulant disrupts Gram negative bacteria by chelating divalent cations that stabilize the lipopolysaccharide coat. Earlier approaches describe EDTA as the most compatible anticoagulant for DNA isolation, however new technologies are now available for effective extraction. We examined the effect of BCT additives: EDTA, acid citrate dextrose solution-A (ACDA), and heparin anticoagulants on *Rickettsia rickettsii* (*Rri*) stability by qPCR and cell culture viability. Blood from 6 donors was spiked with 1.8x10^4 copies *Rri*/mL. For qPCR, 200µL aliquots were sampled on days 0, 1, 3, 7, 10, 14, 21, and 28. For viability, white blood cells (WBCs) were prepared on days 7, 14, 21, and 28. Vero E6 cells were inoculated with 50µL of WBCs and monitored for CPE 4-13 days post inoculation. Results show heparin consistently yielded the highest *Rickettsia* quantities by qPCR, whereas ACDA exhibited the longest duration of viability by culture. Overall ACDA was most stable and consistent by qPCR and culture, while EDTA exhibited the most degradation. Upon closer observation, heparin showed a slight increase of *Rri* followed by stabilization up to day 14 (ANOVA; p ≤ 0.001), while this was not observed with EDTA or ACDA. Further studies are necessary to determine the cause of this increase. Ultimately, ACDA and heparin may be more effective additives for the stability of *Rri* in blood.
Matthew T. Robinson – Factors affecting the successful *in vitro* isolation of rickettsial organisms from clinical samples

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In Lao PDR, rickettsia infections are responsible for 27% of fevers in adults with negative blood-cultures. Serological analysis shows that *Orientia tsutsugamushi* accounts for 14.8%, whilst *Rickettsia typhi* accounts for 9.6%. As part of the diagnostic support and research within Mahosot Hospital Microbiology Laboratory (Vientiane, Lao PDR), *in vitro* *O. tsutsugamushi* and *R. typhi* isolation is attempted from blood samples from patients with suspected rickettsial infections. We have reviewed the isolation and clinical data collected over a period of seven years to identify factors leading to successful isolations, with the aim of optimizing and improving rates of isolation. Factors examined include patient demographics (age, gender, location), clinical history (duration of fever), admission data, diagnostic investigations (IFA, RDT, PCR), sample data (time between blood draw and infection, blood fraction used) and isolation data (staff member, IFA confirmation, duration of culture). Between 2008 and 2014, 3,227 clinical samples were sent for *in vitro* isolation. A total of 256 culture positives were obtained (7.9%), 228 were *O. tsutsugamushi* (89.1%) and 23 were *R. typhi* (9.0%). Review of the clinical and isolation data has highlighted a number of factors that appear significant for the successful isolation of organisms. In reviewing these factors we have developed a number of recommendations that should enhance our ability to isolate rickettsial organisms from clinical samples. Although these factors may be particular to Lao PDR and the microbiology laboratory here, these factors should be considered wherever *O. tsutsugamushi* and *R. typhi* isolation is carried out in other laboratories.
Human pathogenic Rickettsia cause mild to severe diseases worldwide. Nonspecific symptoms at the early stage of illness confounds clinical diagnosis. Timely diagnosis is essential for effective treatment especially in Rocky Mountain spotted fever (RMSF) infection, where a delay in doxycycline administration correlates with more severe outcomes and death. Intrinsically low bacteremia reduces the accuracy of laboratory diagnosis, and at the lower limits of detection (LoD) the sensitivity of our real-time PCR (PanR8) at ~9 copies (5µL)/reaction requires ~1,800 genome copies/mL blood. We developed a Pan-Rickettsia reverse transcriptase real-time PCR assay (PanR6) targeting both rRNA and rDNA in total nucleic acid (TNA). With high analytical specificity, PanR6 detects 16 Rickettsia species and excludes 15 near neighbors and >50 bacteria, protozoa, fungi and human DNAs. In fatal and nonfatal rickettsioses cases, 23 positive clinical samples and 7 negative samples were tested by PanR8/DNA and PanR6/TNA. All 9 RMSF fatal case samples were positive by both methods. Nonfatal rickettsioses patient blood specimens (7) were positive, 4 by both methods and 3 by PanR6/TNA only. In fatal case samples (blood, serum, and 6 tissue types) the PanR6/TNA assay Ct values averaged 7.6 (5.72-9.33) <PanR8/DNA; and in blood from nonfatal cases Ct values averaged 5.1 (3.68-6.33) <PanR8/DNA. Ten-fold serial dilutions showed increased detection up to 100,000X the PanR8/DNA LoD. Positive and negative predictive values (NPV) for PanR6/TNA are 100%; 30% greater than the PanR8/DNA NPV of 70%. Increased sensitivity and accuracy of rickettsial real-time PCR detection in patient samples represents an important step towards more effective laboratory diagnosis of rickettsioses.
Since 1998, when the first rickettsial genome, that of *R. prowazekii*, was published, more than 100 rickettsial genomes, including 92 from 28 validly published species, were sequenced. Access to these genome sequences has provided an essential insight into their evolution and physiology, with the discovery of unexpected characteristics such as a reductive evolution by progressive gene degradation (1.5 → 1.1 Mb), mainly of ATP, amino-acid, LPS and cell wall component biosynthesis; a paradoxical link between genomic reduction and increased pathogenicity; the proliferation of genetic elements: rickettsial palindromic elements, plasmids, type IV secretion system, tetratricopeptide and ankyrin repeat motifs, paralogous gene families (*sca, spoT, tlc, proP, ampG*), toxin-antitoxin modules; the presence of highly conserved non coding regions; the presence of an active conjugation system; the evidence of genetic exchange with other amoeba-associated bacteria by lateral gene transfer. The genomic analysis of rickettsial genomes suggested that current *Rickettsia* species have evolved from a free-living ancestor that adapted to intracellular life into a eukaryote, possibly amoebae, within which they exchanged DNA with other intra-amoebal pathogens and progressively underwent a reductive genomic evolution associated to increased virulence possibly by loss of regulation.
The development of a formal order analysis (FOA) [Gumenuk et al., 2013] allowed to construct a classification of 36 genomes of representatives Rickettsiaceae family [Shpynov et al., 2015]. Recently FOA has been extended by new tools «Locality-sensitive hashing» (LSH), «Map of genes» (MG) [Pozdnichenko et al., 2016] and «Matrix of similarity» (MS) [Gumenuk et al., in press] for a more in-depth study of the structure of rickettsial genomes (chromosomes, plasmids). All genomes have been imported from the database GenBank NCBI (USA): www.ncbi.nlm.nih.gov/genome. We have extended the classification by adding the genomes of seven Rickettsia spp. The new classification confirmed and supplemented the previously constructed one, based only on one characteristic of the order - the average remoteness (g), by determining the position of Rickettsia africa ESF-5, R. heilongjiangensis 054, R. monacensis str. IrR/Munich, R. montanensis str. OSU 85-930, R. raoultii str. Khabarovsk, R. rhipicephali str. 3-7-female6-CWPP and Rickettsiales bacterium Ac37b. The MG demonstrated the complete genomes and their components in a graphical form. Rickettsial genomes are placed (classified) according to the index g on the X axis, and their components (coding and non-coding sequences) sited by depth index (G) on the Y axis. The MS was applied for an in-depth classification to sub-taxonomic category of the strain within the species of R. rickettsii (11 str.) and R. prowazekii (10). MS determines degree of homology of complete genomes by pairwise comparison of their components and identification of identical and similar on composition of nucleotides. The function of the local characteristics of order («Sliding window») was used for verification of completely matching and high homology pairs of components [Pozdnichenko et al., 2014]. Numerical characteristics of order can be used for compact representation and LSH of nucleotide sequences of complete genomes. A new approach «Genomosystematic» is proposed for the study of complete genomes and their components through the development and application of FOA tools. Its tasks include the development of principles for the classification of microorganisms, based on the analysis of complete genomes and their annotations.
Rickettsia philipii is an emerging human spotted fever group pathogen which was first isolated from ticks in 1960's and then implicated as a cause of human illness in 2009. *Rickettsia philipii* is vectored by *Dermacentor occidentalis* and causes Pacific Coast tick fever. The disease is milder than classic Rocky Mountain spotted fever, and presents with an eschar but may require hospitalization in pediatric patients. This presentation will discuss the unique features of *R. philipii*, including its phenotypic, genetic and genomic differences from *R. rickettsii*, its nearest relative, and compare classic and recently established isolates of this novel pathogen.
Japanese spotted fever (JSF) is a condition characterized by a rash that has early macules, and later, in some cases petechiae is detected. It is caused by Rickettsia japonica. The diseases have been increasing in recent years. Tetracycline and new quinolone are used as an empiric therapy, however, exemplary therapy based on evidences are not established. Some patients didn’t recover due to DIC, MOF (Multiple Organ Failure) and bacteremia. The standard therapy to treat JSF is necessary to prevent the advancing in severity. Experimentally tetracycline, new quinolone and the combination of both agents are effective to inhibit releasing of cytokine and chemokine in inflammatory state. Combination therapy has not been proven to give a remarkable effect on JSF compared to monotherapy. This time we analyzed JSF, which were reported previously, in the grades of death, severe and mild cases. Considering about therapy, we examined the severity including febrile state duration. We looked up 15 reports written in English and 33 cases reported in Japanese. Results were as the followings. 1) Cases of JSF; Cases consisted of 4 death cases, 21 severe cases and 40 mild cases. The median age was 64.5 (0-84 y.o.). Regions of occurrences were Hyogo Prefecture (13 cases), Shimane Prefecture (9 cases) and Tokushima Pref. (7 cases). Those areas are located in the west of Japan. Using the clinical severity scoring, we calculated the score in each case. 2) Analysis of therapy; Mono-therapy by minocycline was effective, showing that 25 mild cases, 15 severe cases and 1 death case. In this analysis, 4 death cases showed the score 6.0. The score in 21 severe cases was 4.7 and that in 40 mild cases was 1.8. 3) Duration of febrile period; From the beginning of the therapy until afebrile state, minocycline monotherapy took 3.8 days and combination therapy took 2.6 days. The data indicated significant difference. In conclusion, it was suggested that combination therapy was more effective than monotherapy in JSF.
Rong Fang – Atg-5 dependent autophagic response in macrophages supports the survival of Rickettsia australis via inhibiting IL-1β secretion

Jeremy Bechelli, Leoncio Vergara, Tetyana P. Buzhdygan, Claire Smalley, Bing Zhu, Sean Bender, Yan Liu, Seungmin Hwang, Vsevolod L. Popov, David H. Walker, Rong Fang

The interactions of autophagy system with rickettsiae, cytosolic-replicating bacteria, have never been well understood. In the present study, we examined whether R. australis interacts with autophagy in mouse bone marrow-derived macrophages (BMMs), and its impact on rickettsial survival in vitro and in vivo. Transmission electron microscopy demonstrated Rickettsia-containing double-membrane-bound vacuoles typical of autophagosomes. By immunofluorescence confocal microscopy, we observed an increased number of LC3 puncta upon infection and co-localization of R. australis with autophagosomes. While lipidated LC3 levels were significantly increased, the levels of SQSTM1/p62 remained unaltered in BMMs upon infection with R. australis. Furthermore, autophagosomes was induced by R. australis without p62 turnover in Atg5 flox/flox BMMs, but not in Atg5 flox/flox Lyz-Cre BMMs, suggesting that the accumulation of autophagosomes by rickettsiae is Atg5-dependent. Concentrations of intracellular R. australis in Atg5 flox/flox BMMs were significantly greater compared to Atg5 flox/flox Lyz-Cre BMMs, accompanied by a reduced secretion level of IL-1β. Enhanced R. australis survival in Atg5 flox/flox BMMs vs. Atg5 flox/flox Lyz-Cre BMMs was abrogated upon treatment with recombinant IL-1β. Along with these in vitro data, bacterial loads in tissues of R. australis-infected Atg5 flox/flox mice were significantly greater compared to Atg5 flox/flox Lyz-Cre mice. Deficiency of Atg5 in macrophages significantly enhanced serum levels of IL-1β in R. australis-infected mice compared to the counterparts. Taken together, our study demonstrated that R. australis accumulates autophagosomes in primary mouse macrophages via Atg5, which facilitates rickettsial survival through inhibiting IL-1β secretion.
Isaura Simões – Host players involved in the interaction between spotted fever group rickettsiae and macrophage-like cells

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Endothelial cells have long been considered the main target cells for rickettsiae. However, several studies have provided evidence of non-endothelial parasitism by rickettsial species with numerous intact bacteria being found within the cytoplasm of macrophages and neutrophils, both in tissues and blood circulation. This evidence has raised debate about the biological role of this interaction during rickettsial pathogenesis. We have recently reported that two members of spotted fever group *Rickettsia* (*R. conorii* and *R. montanensis*) have completely distinct intracellular fates in human THP-1-derived macrophages (Curto P. et al. (2016) Front. Cell. Infect. Microbiol. 6:80). Although the interaction of rickettsiae with endothelial cells is a process relatively well studied, little is known about the interaction of rickettsial species with macrophages. In this work, we employed a pharmacological study to start understanding the host proteins involved in the rickettsial entry process into macrophages. Using PMA-differentiated THP-1 cells and *R. conorii* and *R. montanensis* as our models of study, we have identified a requirement of actin polymerization, receptor and non-receptor tyrosine kinase proteins and Pak1 for rickettsial entry into macrophages. Previous reports have demonstrated that PI3K is required for rickettsial invasion in Vero cells. However, our results suggest that PI3K is not required for rickettsial entry into macrophage cells, thereby suggesting that different pathways could be involved in rickettsial uptake in this cell type. Surprisingly, amiloride Na⁺/H⁺ exchangers inhibitors (DMA and EIPA) - which are known to be the main diagnostic test for macropinocytosis - were able to block rickettsial association with macrophage cells, thus suggesting a macropinocytosis-related pathway as a possible route of entry of rickettsiae in macrophages. These results, and our ongoing work involving high throughput proteomics analysis of rickettsiae-macrophage interaction, will provide a deeper understanding on how differences in bacterial interactions with distinct cellular host factors contribute for species-specific patterns of rickettsial cellular tropism and pathogenicity.
Monika Danchenko – European isolate of *Rickettsia felis* from a tick

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The isolation of a native strain provides the possibility to study the biological properties, antibiotic susceptibility and the epidemiology of rickettsiae. Shell-vial culture assay is a powerful tool to detect *Rickettsia* sp. Although *Ctenocephalides felis* has been designated as the main vector of *R. felis*, various arthropods, fleas, ticks, mites and lice have been associated with this bacterium. It has been reported, that different microorganisms can be obtained via a blood meal from an infected rodent, or co-feeding of ectoparasites, and that cross-reaction can occur under natural conditions, as different vectors share rodents as reservoir hosts. We succeeded in isolation of *Rickettsia* sp., namely *R. felis* from a host-seeking nymphal *Ixodes ricinus*, the most frequent tick in Slovakia. The isolation was performed on XTC-2 cells at 28°C by the shell-vial technique. Evaluation of growth properties and propagation was made on both XTC-2 and Vero cell lines. We beheld apparent cytopathic effect in infected host cells at the earliest on 6 dpi. *Rickettsia felis* was observed microscopically. New *R. felis* isolate IR16 was purified by isopycnic density gradient centrifugation. Fragments of the genes *gltA*, *ompB*, *sca4*, *rflE* and *rrs* were sequenced and compared with the corresponding sequences of the type strain URRWXCl2. Hereby we describe a unique *R. felis* isolate, yielded from a tick, and deliver an evidence of a possible circulation of this fastidious rickettsia in Slovakia.

Acknowledgement. This study was financially supported by the projects VEGA 2/0005/15 from the Scientific Grant Agency of Ministry of Education and SAS, APVV-0280-12.
Three rickettsial genotypes detected in mosquitoes from the republic of Korea

Allen L. Richards – Three rickettsial genotypes detected in mosquitoes from the republic of Korea

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Rickettsia species are Gram-negative obligate intracellular bacteria that are associated with a diverse range of invertebrate hosts and can infect various vertebrate hosts including humans resulting in diseases ranging in severity from mild to life threatening. The invertebrate hosts the commonly transmit the causative agents of rickettsial diseases include ticks, mites, lice and fleas, but the list of invertebrate hosts continues to grow incorporating mosquitoes. The mosquitoes could emerge as one of the more important invertebrate hosts if they are able to transmit rickettsial pathogens as they do other bacterial and viral pathogens and parasites throughout the world. Recent studies have implicated Anopheles gambiae as a potential vector of the pathogen, Rickettsia felis. Here we report that a new metagenome sequencing effort identified rickettsial sequence reads in mosquitoes from the Republic of Korea. The detected rickettsiae were characterized by quantitative real-time PCR (qPCR) assays and sequencing of rrs, gltA, ompB, sca4 and the 17kDa antigen genes. Three unique rickettsial genotypes were detected (Candidatus sp. A12.2646, Candidatus R. sp. A12.2638 and Candidatus R. sp. A12.3271), from Mansonia uniformis, Culex pipiens, and Aedes esoensis, respectively. The importance of this discovery could be significant not only scientifically but also in terms of public health.
Jane E. Koehler – Molecular adaptation of Bartonella to its human and louse niches

Henriette Macmillan, David Dranow, Sally Lyons-Aubott, Gina Borgo, James Fairman, Stephanie Huezo, Donald Lorimer, Bart Staker, Robin Stacy, Stephanie Abromaitis, Thomas Edwards, Peter Myler, Jane Koehler*

The general stress response (GSR) is a widely conserved response utilized by bacteria to survive under extreme environmental conditions. Bartonella quintana (BQ) occupies two distinct niches: the bloodstream of the human host (37°C, restricted hemin levels), and the gut of the human body louse vector (28°C, toxic hemin levels). We previously identified an extracytoplasmic function sigma (σ) factor, RpoE, which is involved in the GSR of BQ. Once in the louse, BQ uniquely activates the GSR in response to the decreased temperature (28°C), and a sensor histidine kinase (BQ-SHK) phosphorylates an anti-anti-σ factor, PhyR. Phosphorylated PhyR then competes for, and removes, the anti-σ factor NepR from the RpoEσ-hus enabling transcription of the regulon necessary for survival under body louse conditions. To identify the structural basis of BQ GSR regulation in the body louse, we solved the crystal structures of the RpoE-NepR complex, the NepR-PhyR complex, and unbound, unphosphorylated PhyR. These crystal structures revealed a dramatic conformational change in PhyR after phosphorylation. The BQ-SHK null mutant was unable to activate the GSR in response to the 28°C stress signal. Our data show that RpoE, BQ-SHK, and the conformational change of PhyR after phosphorylation all have critical roles in the adaptive response of BQ to low temperature stress in the body louse arthropod vector.
Diana G. Scorpio – *Bartonella* spp. - Public Health Risk for Transfusion Patients due to Detection in Stored Blood Products

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Transfusion medicine is a critical component of medicine, and in developed countries strict protocols minimize transmission of infectious agents. Depending on the country, World Health Organization recommendations are used (HIV, Hepatitis B and C, syphilis, malaria). The consequences of inadvertently infecting at-risk patients, such as those with immunosuppression can result in unacceptable co-morbidities, even death. There is increasing concern over transfusion-transmitted zoonotic agents, such as *Babesia*, trypanosomes, and tick-borne agents; however, there are no public health measures in place to screen for *Bartonella* at this time. Neither the American Association of Blood Banks (AABB) nor the Food and Drug Administration (FDA) provide guidance for *Bartonella* screening of donor blood in the USA. Research regarding *Bartonella* infections and chronic disease in humans is lagging. Infections caused by *Bartonella* can be subclinical or mimic other chronic diseases for which etiology is not easily identified. Furthermore, the organism is difficult to culture and antibody tests suggest exposure but do not confirm active infection. In Brazil, *Bartonella* spp. were found in stored blood from donor clinics, using alternative isolation procedures and sensitive PCR methodologies. Both *B. henselae* and *B. clarridgeiae* were detected in 3.2% of stored blood units. Neither adverse consequences of transfusion-transmitted bartonellosis nor risk factors associated with this infection have been well-studied. Survey of 500 blood donors in Brazil revealed *B. henselae* and *B. clarridgeiae* bloodstream infections were significantly associated with cat contact (odds ratio: 3.4) and history of tick bite (odds ratio: 3.7). In addition to flea transmission, tick bite transmission of *B. henselae* is proposed but is controversial. While concrete guidance regarding transmission of vector-borne agents, including *Bartonella* species have not been formulated, the identification of risk factors should therefore be considered in pre-donation screening procedures before blood donation.
Bartonella genus includes small, fastidious, Gram-negative, facultative intracellular pathogens with a unique intraerythrocytic lifestyle. These bacteria are adapted to two quite different environments: the intestinal tube of hematophagous arthropods and the bloodstream of the mammalian host. The epidemiological description of different bartonellae are based usually on the detection of bacteria in the blood of the mammalian host or in the arthropods (or both). Over the past 20 years, there has been a rapid increase in the number of Bartonella species, with around 35 new species, however, bartonellae from African continent stay still mostly uninvestigated.

Our team studied the Bartonella bacteria presence in hosts and vectors in West African countries, mostly in Senegal. We have succeeded in isolation of around 50 Bartonella strains from mammals and arthropods, including at least 5 new species: B. massiliensis and B. senegalensis from Ornithodoros sonrai soft ticks, B. raoultii, B. mastomydis and B. saheliensis from Mastomys erythroleucus and Gerbilliscus gambianus. We identified that the most common rodent in Dakar, Gambian pouched rat Crycetomys gambianus, is infected by B. elizabethae-like bacterium.

Studying the causes of acute febrile diseases in Senegal, we have screened 1800 DNA samples extracted at place in rural out-patient departments (dispensaries in Dielmo and Ndiop villages). We have detected 20 patients (1.1%) with the DNA of B. quintana in the blood. Moreover, 10 cases were detected during 4-months period in June-October 2014 in the same village (Ndiop). During this outbreak we arrived to isolate two identical B. quintana strains, one from the patient, another from the head louse of the same patient after the treatment. The present outbreak was associated with the cytB A group head lice widely distributed in the village. No body lice were detected in any of 20 patients and, according to the village elders, body lice are not seen in the village since more than 50 years.

We believe that here we documented the head lice transmitted trench fever outbreak that may change the epidemiological paradigm of this disease.
Algimentas Paulauskas – Infections with *Bartonella* spp. in free ranging cervids and deer keds (*Lipoptena cervi*) in Southern Norway

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*Bartonella* species are Gram-negative, facultative intracellular bacteria. The bacteria are hemotrophic, arthropod-borne and can cause long-term bacteremia in mammalian hosts. The predominant arthropod vectors and the mode of transmission for many novel *Bartonella* species remain elusive or essentially unstudied. The aim of the present study was to investigate the prevalence of *Bartonella* infection in cervids and deer keds and to characterize the isolates in order to evaluate a possible transmission route. A total of 183 samples of spleen from roe deer, red deer and moose were collected through the hunting season in southern Norway. Wingless deer ked imagines were collected from the skin of the carcasses. PCR and sequencing of the partial *gltA* gene and 16S-23S rRNA intergenic spacer region (ITS) were applied for *Bartonella* identification. *Bartonella* spp. was detected in 49 out of 59 pools of adult wingless deer keds, in 3 out of 40 spleen samples of roe deer, in 7 out of 25 of red deer and in 31 out of 118 of moose. Phylogenetic analysis based on *gltA* gene demonstrated two distinct groups of sequences which showed 98-96 % similarity with *B. bovis* (moose samples) and 99-93 % similarity with *B. schoenbuchensis* (red deer and deer ked samples from red deer). Phylogenetic analysis of ITS region sequences derived from roe deer revealed 97% similarity with *B. bovis*, while sequences derived from red deer showed 99-97% similarity with *B. schoenbuchensis*, *B. chomelii* and *B. capreoli*. 
David A. Jaffe – Detection and seroprevalence of *Bartonella henselae* in small Idian mongooses (*Herpestes autopunctatus*) from Granada island

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Domestic cats (*Felis catus*) are the natural reservoir of a few *Bartonella* species, including *Bartonella henselae* the agent of cat-scratch disease. Many mammals are established hosts for these bacteria. Indian mongooses (*Herpestes auropunctatus*), as domestic cats, belong to the *Feliformia* phylogenetic suborder, and have been reported as a possible host for *B. henselae* in southern Japan. Confirming the prevalence of *Bartonella* in mongooses from the Caribbean could identify them as vectors for human transmission, and the risk involved with mongoose-human contact in the New World. No study has previously looked for *Bartonella*’s presence in mongooses from Grenada Island. We used immunofluorescence, polymerase chain reaction, and sequencing techniques to establish the presence of *B. henselae* in 171 mongooses from all six parishes in Grenada. Almost a third (32.3%, 54/167) of the mongooses were seropositive for *B. henselae*, and 13 (36.1%) of the DNA extracted from blood pellets (n=36) were PCR positive for the gltA (citrate synthase) gene. However, only one of 136 DNA samples extracted from tissue (a mixture of spleen, liver and mesenteric lymph nodes) was PCR positive for *Bartonella*. Sequencing confirmed that all sequences were identical to *B. henselae* genotype I, as previously reported from Japan. Sex or parish of origin were not identified as risk factors for seropositivity. This study confirms the role of Indian mongooses as a natural reservoir of *B. henselae* in the New World.
Scrub typhus is a neglected, potentially fatal zoonosis that significantly impacts global health. The etiologic agent is Ori
tedia tsutsugamushi, an obligate intracellular rickettsial bacterium that infects leukocytes and endothelial cells. The ability of O. tsutsugamushi to replicate in the cytosol of diverse host cell types, including professional phagocytes, suggests that it evolved to inhibit processes that occur in response to intracellular infection and to exploit metabolic pathways to satisfy its nutritional virulence requirements. The mechanisms by which it does so have remained elusive. The ankyrin repeat is the most common protein-protein interaction motif in nature. Orientia boasts one of the largest microbial arsenals of ankyrin repeat-containing proteins and expresses its entire repertoire of these Type I secretion system effectors during infection. Studying Orientia Anks should therefore provide an effective means for gaining insight into the diverse host cellular processes that the bacterium co-opts or manipulates. Indeed, by doing so we have thus far determined that Orientia and a subset of its Anks modulate SCF1 ubiquitin ligase assembly, the unfolded protein response, ER-associated degradation, protein secretion, and Golgi-to-ER retrograde trafficking. We recently discovered that O. tsutsugamushi antagonizes Nf-κB dependent transcriptional activation, the central initiating cellular event in the antimicrobial response, by preventing nuclear accumulation of Nf-κB. We linked this phenomenon to two Ank effectors and dissected the mechanism by which they modulate Nf-κB. Doing so revealed an unprecedented mechanism by which the effectors interact with both the p65 subunit of Nf-κB and exportin 1 to mediate nuclear export of Nf-κB. This lecture will provide an overview of the O. tsutsugamushi Ank arsenal and define in detail the novel means by which the Anks negatively regulate Nf-κB.
Paul Newton – Clinical trial of doxycycline and azithromycin therapy in murine typhus therapy¹

Background. Murine typhus, or infection with Rickettsia typhi, is a global but neglected disease with no randomised clinical trials to guide antibiotic therapy. Doxycycline is commonly used but without objective evidence of course duration. Azithromycin is a possible alternative, especially for pregnant women.

Methods. A prospective, open, randomised trial in non-pregnant consenting adults with rapid diagnostic test evidence for infection with uncomplicated murine typhus at two hospitals in Vientiane, Lao PDR, with three treatment regimes: seven days doxycycline (D7), 3 days doxycycline (D3) and 3 days azithromycin (A3). Fever clearance time, area under the fever-time curve, frequency of treatment failure rate and relapse rate were the main outcome measures.

Results. Two hundred and sixteen patients (72 patients in each arm) were randomised 2004-2009: 158 (76.4%) patients had confirmed murine typhus, without evidence of dual infections, including 52 (25.1%) with PCR lone-infection evidence. Mean (95%CI) total doses were 27.5 (26.0-29.0) and 14.0 (13.1-15.0) mg/kg body weight for D7 and D3 patients, respectively, and 18.9 (18.1-19.6) mg/kg for A3. All patients survived to discharge. There were 1.4, 4.2 and 22.5% failures in the D7, D3 and A3 treatment groups, respectively (P<0.001). Median fever clearance time was significantly longer for patients in the A3 group. The area under the curve for temperature and time to fever clearance time or maximum time in hospital was also significantly longer for those R. typhi PCR confirmed. The median (range) follow up duration was 208 (2-666) days and 26 (12.0%) patients returned with fever. No patients returned with PCR confirmed relapse.

Discussion & Conclusions. This trial suggests that azithromycin is inferior to doxycycline for the oral therapy of uncomplicated murine typhus in Laos but that three days doxycycline is appropriate for treating uncomplicated murine typhus in adults. Use of azithromycin in pregnant women should be reconsidered for murine typhus but there are no clear alternatives. Acquired azithromycin resistance in other human pathogens is mediated by the ribosomal target 23s rRNA and urgent investigation of genomic and phenotypic markers of azithromycin resistance is needed.
Scrub typhus is a vector-borne zoonotic disease that can be life-threatening. There are no licensed vaccines, or vector control efforts in place. Despite increasing awareness in endemic regions, the public health burden and global distribution of scrub typhus remains poorly known.

Scrub typhus is a leading cause of treatable non-malarial febrile illness in prospective Asian fever studies. Sero-epidemiological data also suggest that Orientia tsutsugamushi infection is common across Asia, with seroprevalence ranging from 9 - 28% (median 22% IQR 19-26). Data from passive national surveillance systems - available for South Korea, Japan, China, and Thailand - outline a substantial apparent rise in minimum disease incidence. Case fatality risks from areas of reduced drug-susceptibility are reported at 12-13% for South India and northern Thailand, respectively. Mortality reports vary widely around a median mortality of 6.0% for untreated and 1.4% for treated scrub typhus. Limited evidence suggests high mortality in complicated scrub typhus with CNS involvement, multi-organ dysfunction and high pregnancy miscarriage rates with poor neonatal outcomes.

Scrub typhus appears to be a severely neglected tropical disease of substantial impact on rural populations. All countries with established surveillance systems report increasing minimum incidence rates in the past decade. Recent reports from South America and Africa suggest a wider distribution outside Asia. However, many limitations on the amount and quality of available epidemiological data, limit conclusions, mathematical modeling or mapping approaches. This presentation aims at putting novel findings, considerations and relevant knowledge gaps into a current perspective.
Scrub typhus, caused by the intracellular bacterium, *Orientia tsutsugamushi* has an estimated incidence of 1 million patients/year and untreated case fatality of approximately 10%. The traditional geographical distribution across South Asia and the Western Pacific is being challenged by cases identified in Chile, the Middle East and possibly Africa. Research during and immediately after WW2 led to much of our current understanding of scrub typhus ecology. Our literature review identified 370 papers investigating *O. tsutsugamushi* in vectors and hosts (excluding humans). Of these, 247 are in English. An additional 53 papers examined general aspects of scrub typhus ecology. Chiggers (mite larvae) of *Leptotrombidium* species are considered the major vector, however there are increasing reports of Orientia in other genera and even in other animals including leeches. The role of rodents and birds in the ecology and distribution of the disease is uncertain. Chiggers are described as biting only once but evidence of mite-feeding behavior are lacking. The concept of “mite islands” – patchy distribution of infected vectors was coined in the 1940s based on variable infection rates among soldiers deployed in scrub-type habitats. Much of the literature utilized diagnostic methods with poor sensitivity and specificity, particularly xenodiagnoses, microscopy and the Weil Felix test. We reviewed the literature to summarise and map studies examining animals and chiggers infected with *O. tsutsugamushi*. We highlight important gaps in our knowledge and suggest that modern tools such as whole genome sequencing and geographical information systems will advance our understanding of this pathogen’s complex ecology.
Scrub typhus, caused by the obligate intracellular bacterium *Orientia* (*O.*), *tsutsugamushi*, is a potentially fatal infection with high endemicity in Southeast Asia, for which only a limited number of antimicrobials is at hand. Increasing reports on cases that are clinically non-responsive to the gold standard treatments highlight the urgent need for novel drug targets. Corallopyronin A (CorA), a compound isolated from the myxobacterium *Corallococcus coralloides*, binds to the switch region of bacterial DNA-dependent RNA polymerase (RNAP) and inhibits RNAP of an increasing number of bacterial species. We showed that CorA inhibited growth of *O. tsutsugamushi* in vitro at a minimal inhibitory concentration (MIC) of 0.078 μg/ml, compared to a 16-fold higher MIC of 0.125 μg/ml found for *Rickettsia typhi*, demonstrating high susceptibility of *O. tsutsugamushi* towards CorA. In vivo, 100 μg CorA was the minimal daily dose with which 100% survival of mice was achieved in the lethal intraperitoneal (i.p.) infection model of BALB/c mice. Two subsequent doses of 100 μg were sufficient to protect from lethal infection. We also investigated whether *O. tsutsugamushi* persistence after treatment was associated with the acquisition of antimicrobial resistance. However, *O. tsutsugamushi* re-isolated >600 days p.i. from i.p.-infected, CorA-treated mice demonstrated MICs identical to the pre-inoculation strains. The absence of resistance mechanisms was further confirmed by sequencing the CorA target regions of RNAP, which showed no mutations. In summary, *O. tsutsugamushi* infections are a highly relevant target for the novel RNAP inhibitor CorA that is effective at low doses and short-term treatments.
Manisha Biswal – Genetic diversity of *Orientia tsutsugamushi* strains from patients in north India

Manisha Biswal, Abhay Kumar, Kamran Zaman, Navneet Sharma, Sunit Singhi

**Background**

Scrub typhus has emerged as a major cause of acute febrile illness in India. The causative agent, *O. tsutsugamushi* has more than 20 antigenic types due to a variable 56-kDa outer membrane protein. It is crucial to know the prevailing antigenic types in India for the success of diagnostic immunoassays and prospective vaccine candidates. The current study was planned to identify the genotypes of *O. tsutsugamushi* circulating in a wide area of north India.

**Materials and methods**

DNA was extracted from whole blood of patients with suspected scrub typhus, between July 2013 and December 2013. A nested PCR was used to amplify a 483-bp region of the 56-kDa antigen gene of *O. tsutsugamushi*. The DNA sequences were aligned using the CLUSTAL V program. A phylogenetic tree was constructed using NJ algorithms and analysed.

**Results**

A total of 93 PCR products were sequenced and analyzed. Boryong-like strains predominated in all states studied (63.4%) followed by Karp-like (23.6%) and Gilliam-like (11.8%). We did not find any Kato-like strains and only one Kawasaki-like strain. Karp like strains showed >99% similarity to TH2033, TH2191, TH2208, Xinjiang & Neimeng strains and Gilliam- like strains showed >99% similarity to Clone ISS -11, Hualien 1, S072.

**Conclusion**

A previous study using PCR from eschars from south India showed a predominance of Kato-like strains but we could not find even a single Kato-like strain. Boryong-like strains predominated in our study. This shows that there is a huge diversity of *Orientia tsutsugamushi* in India. Boryong strains should be included in diagnostic assays as well as vaccines for scrub typhus, especially for north Indian populations.
Orientia tsutsugamushi is the causative agent of scrub typhus, a potentially deadly infection threatening over one billion people. An obligate intracellular bacterium that is auxotrophic for amino acids, Orientia modulates host cell functions to support its growth. How it does so is poorly understood. The cytoprotective unfolded protein response (UPR) relieves endoplasmic reticulum (ER) stress by promoting ER-associated degradation (ERAD) of misfolded proteins. Here, we show that Orientia temporally modulates ER stress and ERAD, benefitting in an amino acid-dependent manner. During early infection ER stress is induced, but wanes by time points that correspond to the Orientia logarithmic growth phase. Pharmacologically inducing ER stress enhances Orientia replication, while inhibiting it hinders Orientia growth in a manner that is rescued by the addition of free amino acids. Conspicuously, during this period of slow bacterial growth and UPR induction, ERAD is inhibited. ERAD function is restored when bacterial proliferation is robust and the bacterium’s need for amino acids would presumably be high. Indeed, prolonged inhibition of ERAD via siRNA knockdown of the ERAD chaperone Bat3 results in an Orientia growth defect similar to that induced by TUDCA. This replication defect is rescued by free amino acid supplementation. These observations reveal a nutritional virulence mechanism whereby Orientia creates a bottleneck at the ER that, when subsequently released, likely generates a pool of free amino acids that benefits bacterial proliferation. These findings hold a potential translational benefit, as pharmacologic inhibition of ER stress could potentially be used to treat scrub typhus.
Stephen Graves – A new vaccine for Q fever

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Q fever (infection with *Coxiella burnetii*) is widespread in Australia, affecting both humans and a range of animal species. An outbreak on an intensive dairy goat farm in south-eastern Australia (Victoria) was detected when goat abortions increased and 24 persons associated with the farm developed acute Q fever. An attempt to vaccinate the goats with "Coxevac" was thwarted when the Australian quarantine authorities refused to allow the importation of this vaccine into Australia. Hence a decision was made to develop a new vaccine for Q fever in goats based on the specific strain of *C. burnetii* causing the outbreak. The "Meredith" (AuQ60) strain of *C. burnetii* was isolated from aborted goat placenta, grown in embryonated chicken eggs, semi-purified and inactivated with 1% formaldehyde. The new vaccine was tested for efficacy with an aerosol challenge model in guinea pigs. While unvaccinated guinea pigs developed 3-4 days of fever, the vaccinated guinea pigs remained afebrile. When used in goats the vaccine was immunogenic, with all goats producing IgM and IgG antibodies. After both the 1st and 2nd vaccine doses in goats there were slight indurations at the vaccination sites but no systemic side-effects were noted. The vaccine has yet to be tested "in the field" on the goat farm. Unfortunately it cannot be compared with "Coxevac" in Australia.
Coxiella burnetii is an obligate pathogen of ruminants, with a worldwide distribution. Miscarriages caused by the resulting “Q fever” not only have economic repercussions for farmers, but also deposit huge amounts of bacteria back into the environment. Here, by adopting a spore-like state, C. burnetii can survive and infect new hosts, including humans. Human infections generally present with flu-like symptoms, however, rare cases can develop chronic Q fever with complications such as chronic fatigue, heart valve infection, or endocarditis. There is no licensed Q fever vaccine in the UK/EU/US. The lipopolysaccharide (LPS) of C. burnetii is the target for current vaccine development as this provides the main determinant of virulence. The proposed pathways for LPS-linked O-antigen biosynthesis are being tested with recombinantly expressed Coxiella ORFs and enzymes from paralogous sugar biosynthetic pathways in Esherichia coli. In particular, comparison to E. coli dTDP-rhamnose biosynthesis is providing clues to the biosynthesis of dihydrohydroxystreptose (DHHS), a sugar thought to be unique to C. burnetii’s O-antigen. Elucidating the pathways to biosynthesis of Coxiella’s unusual O-antigen sugars provides the basis for generating a recombinant O-antigen fragment for use in a glycoconjugate vaccine.
Elodie Rousset – Harmonization of PCR-based diagnosis for improved quality of data: the example of the French network of Q fever surveillance in ruminants

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Many Q fever diagnostic methods have been developed. Nevertheless, the characterization of their performances is often insufficient. Moreover, a harmonization is expected to ensure quality of data produced by a laboratories network. In France, a first major program was conducted in ten départements on the epidemiological situation of Q fever in sheep, goats and cattle. Accordingly, the French National Reference Laboratory (NRL) coordinated the works leading to the production of reliable and comparable results from the ten corresponding veterinary diagnostic laboratories (VDL), mandated by the Ministry of Agriculture, Agrifood and Forestry (MAAF). The presentation will focus on the real-time quantitative PCR (qPCR) methods used for Q fever abortive diagnosis. The steps were their validation, adoption and then monitoring and maintaining performances. First, the needs of the qPCR methods were established with regards to the expected diagnosis application. Consequently, performance characteristics and validation assays were defined according to the guidelines of the French norm U47-600 (AFNOR) and using the NRL reference materials. The same performances were therefore validated for seven analytical processes by two kit manufacturers and one VDL. Second, method adoption assays were achieved in each of the mandated VDL in order to check the proficiency before routine analysis. Especially, the laboratories had to obtain quantitative measures (number of bacteria per mL) within the established accuracy limits. Third, the NRL led a regular monitoring through each laboratory control chart of quantitative data, based on the bias of the reproducibility control. This approach was appropriate to deal with a new analytical method to be implemented and tested within a laboratories network. Indeed, an inter-laboratory proficiency testing program would not have allowed adjustments throughout the program. The results of adoption assays as well as control charts showed the consistency of the results both at the intra- and inter-laboratory levels. The data of the study were also used to identify a cheaper PCR method for Q fever abortive diagnosis. This latter, named “real time PCR relative to a threshold control”, could be used for the Q fever national program next phase, i.e. a voluntary differential diagnosis of abortive diseases in ruminants based on a harmonised protocol.
Q fever is an abortive disease of ruminants essentially transmitted by dusts and aerosols contaminated by parturition products infected with *C. burnetii*. Although environmental samples have occasionally been screened for *C. burnetii*, the relationship between the detection of the bacterium in the environment of ruminant farms and the occurrence of abortions remains poorly understood. In this study, we investigated dust samples from cattle, sheep, and goat farm buildings with the aims to (1) estimate *C. burnetii*'s detection frequency and bacterial loads in the environment and (2) determine whether this environmental contamination is associated with series of abortions attributed to Q fever. The study was performed within the framework of the French platform for animal health surveillance considering 113 ruminant herds with (n=60) or without (n=53) Q fever-related abortions. Dust was sampled using swab cloths and tested by a quantitative PCR method targeting the IS1111 gene. *C. burnetii* DNA was detected in about half of the buildings investigated. Bacterial loads were occasionally very high, with levels sometimes as high as in infectious materials such as placenta and aborted fetuses. Overall, we found that the probability to detect *C. burnetii* DNA was higher in small ruminant farms (compared to cattle farms) and in herds whose series of abortions were attributed to Q fever (compared to those not attributed to Q fever). Further studies about the diversity and the viability of the bacteria detected in these environmental samples are now needed to fully assess the impact of our results in terms of public and animal health.
Although multiple efforts worldwide have been initiated to shed light into the tricks of C. burnetii in pathogenesis and virulence, it is still not really understood how this zoonotic pathogen exists in so different morphological, immunological and clinical features. Here we present our genomic comparison between the virulent phase I strain Nine Mile RSA493 and its attenuated phase II variant, called Clone 4/RSA439. The results are mandatory for the understanding of pathogenicity of C. burnetii. Therefore, we resequenced RSA493 with PacBio and de novo sequenced RSA439 with a combination of PacBio and Illumina HiSeq. The resulting data was assembled with HGAP3 and polished with Illumina reads. Afterwards, whole genome alignment with the public reference sequence and curation took place. Finally, a whole genome comparison of corrected RSA493 and RSA439 was carried out. Additionally, multiple sequence alignments of all regions containing mutations were performed across all sequenced C. burnetii genomes to determine the conservation of the variations. Beside the large known deletion of 25 kb, we identified 26 Clone 4 specific mutations, including two candidate missense SNPs in two different proteins as well as one deletion of 245 bp within an uncharacterized membrane protein (CBU_0918). Conservation analysis showed that this protein was also deleted in some other virulent strains (e.g. Q212, RSA331, Dutch outbreak strains), probably in the context of reductive evolution. Hence, this variation could not explain the pathogenic difference. But a small deletion causing the loss of one amino acid in CBU_0533 is probably virulence associated because this protein is the orthologue of wecA, which has been knocked-out in S. typhimurium to make them avirulent. Focusing our further analysis and studies now on the remaining mutations will be the next – maybe final – step in unrevealing the pathogenic secrets of C. burnetii.
Coxiella burnetii, an obligate intracellular parasite with a worldwide distribution, is the causative agent of acute and chronic Q fever in humans and abortion in ruminants and especially cattle which are considered as the main source for human infection. The aim of this study was the Genotyping of *Coxiella burnetii* from dairy cows in northern of Algeria than to evaluate the genetic characterization of circulating *C. burnetii* DNA samples amplified. To do this, a total of 77 placental tissue fragments were collected from dairy cows (73 fragments sampled after abortion and 04 after natural calving to be used as negative control) during a period of two years from January 2013 until March 2015. Placental samples were kept in Alcohol 70 % in sample tubes.

Samples were firstly screened by quantitative real-time polymerase chain reaction (qPCR) targeting the gene IS1111 and the gene IS 30 A. 14 / 73 (19, 17 %) wells showed a positive results 9/73 (12, 32 %) from Blida and 5/73 (6, 84 %) from Medea. The positive qPCR amplicons were subsequently tested for MST (Multispacer sequence typing) to determine the genotypes of *C. burnetii*, the spacer regions in the *C. burnetii* genome that exhibits higher variation for differentiating the genotypes (Cox2, Cox5, Cox18, Cox37, Cox56, Cox57, and Cox61 COX 62) were selected. This study demonstrated the presence of *C. burnetii* genotype (MST 20 in Blida and MST 49 in Blida) and the importance of cattle in the epidemiology of Q fever in the northern central of Algeria.

**Keywords**
*Coxiella burnetii*, IS1111 gene, dairy cows, placental tissue, Q fever.
P3-2) Chien-Chung Chao – Metabolic profiling of Orientia tsutsugamushi infected mouse serum from a chigger-fed mouse model

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Background: Scrub typhus is an acute zoonosis caused by the obligate intracellular Gram negative bacterium, Orientia tsutsugamushi (OT). Previous studies have shown mRNA expression profile changes following infection by OT in an IP challenged mouse model. To better understand the host response elicited by natural infection by chigger feeding, we exposed ICR mice to OT-infected chiggers and examined the biochemical profiles of their serum over several post-infection time points.

Methods: ICR mice were infected with either individual naïve Lc chiggers (i.e. not infected by OT) or OT-infected Lc chiggers. Serum was isolated from the infected mice at 6h, 12h, 1 day, 2 day, 4 day, 7 day and 10 day post infection and from the sham-infected animals at the 6 hour and 10 days post infection time points. Metabolites were extracted from the serum samples and analyzed by UPLC-MS/MS. The resulting ion features were matched to a library of chemicals standards for identification and quantification (Metabolon, Inc.). Biochemicals that differed significantly between the experimental groups were determined using Welch's two sample t-tests. P-values ≤0.05 were considered statistically significant.

Results: A number of biochemicals linked to immune function (e.g., itaconate, mesaconate, methylsuccinate, kynurenine, and histamine) were found to be significantly altered. These did not become particularly apparent, however until late in the time course (days 7-10). Nevertheless, several metabolites linked to energetic pathways and stress were found to be altered in the animals throughout the time course. The infected mice also differed from the sham-infected mice with regards to food intake, lipid and carbohydrate metabolism, amino acid homeostasis, and nucleotide utilization.

Conclusions: Global untargeted metabolomics revealed significant differences in the biochemical profiles of the sham-infected and OT-infected mice. These findings, importantly, provide a platform for further understanding the host responses associated with chigger-borne OT infections.
Orientia tsutsugamushi, an obligate intracellular pathogen is the causative agent of scrub typhus, an acute febrile disease with diverse clinical presentations. Scrub typhus is transmitted by infected Leptotrombidium mites and is irregularly distributed across endemic regions of Asia, Australia, and the western Pacific. Recent reports indicate an expansion in scrub typhus cases to areas of the Middle East, South America, and Africa. Previous work to understand population genetics in O. tsutsugamushi has been based on sub-genomic sampling methods and limited whole genome characterization of single genomes. This study compared 40 genomes from geographically dispersed areas. The analysis confirmed patterns of extensive homologous recombination likely driven by transposons, conjugative elements, and repetitive sequences. High rates of lateral gene transfer among O. tsutsugamushi genomes appear to have effectively eliminated a detectable clonal frame, but not the ability to infer evolutionary relationships and phylogeographic clustering. Unlike other highly recombinant species where the uptake of exogenous DNA largely drives genomic diversity, the pan-genome of O. tsutsugamushi is driven by duplication and divergence more than any other bacterial species. The near unparalleled evolutionary characteristics of O. tsutsugamushi expand our present understanding of bacterial evolutionary mechanisms while highlighting limitations of existing comparative genomics tools when dealing with highly recombinant and duplicated genomes.
Orientia tsutsugamushi is an intracellular pathogen that replicates in the cytosol of professional phagocytes and endothelial cells to elicit scrub typhus, a potentially fatal disease that affects one million people annually. Activation and nuclear translocation of the transcription factor, NF-κB, is the central initiating cellular event in the antimicrobial response. Whether and how Orientia modulates NF-κB are poorly understood. We discovered that nuclear accumulation of the NF-κB subunit p65 is inhibited in Orientia infected HeLa cells and murine bone marrow-derived macrophages, even in the presence of the NF-κB activating cytokine, TNFα. This results in downregulation of NF-κB dependent transcription. Orientia modulates p65 subcellular localization, but does not degrade it. Additionally, the bacterium does not alter degradation of NF-κB inhibitor, IκBα. Rather, Orientia exploits exportin 1 to promote nuclear export of p65, as this phenomenon is blocked by an exportin 1 inhibitor, leptomycin B. Two Orientia ankyrin repeat-containing effectors (Anks) are linked to the bacterium’s ability to modulate NF-κB, as both translocate to the nucleus, abrogate NF-κB activated transcription, and pronouncedly reduce TNFα-induced p65 nuclear accumulation in an exportin 1-dependent manner when ectopically expressed. Co-immunoprecipitation experiments demonstrate that both are capable of interacting p65 and exportin 1. Overall, these data reveal a novel mechanism by which O. tsutsugamushi Anks co-opt exportin 1 function to promote nuclear export of NF-κB and thereby antagonize a critical arm of the antimicrobial response.
Scrub typhus is a mite-borne febrile disease caused by *O. tsutsugamushi* infection. Recently, emergence of scrub typhus has attracted considerable attention in several endemic countries in Asia and the western Pacific. In addition, the antigenic diversity of the intracellular pathogen has been a serious obstacle for developing effective diagnostics and vaccine. To understand the evolutionary pathway of genotypic diversification of *O. tsutsugamushi* and the environmental factors associated with the epidemiological features of scrub typhus, we analyzed sequence data, including spatiotemporal information, of the *tsa56* gene encoding a major outer membrane protein responsible for antigenic variation. A total of 324 *tsa56* sequences covering more than 85% of its open reading frame were analyzed and classified into 17 genotypes based on phylogenetic relationship. Extensive sequence analysis of *tsa56* genes using diverse informatics tools revealed multiple intragenic recombination events, as well as a substantially higher mutation rate than other house-keeping genes. This suggests that genetic diversification occurred via frequent point mutations and subsequent genetic recombination. Interestingly, more diverse bacterial genotypes and dominant vector species prevail in Taiwan compared to other endemic regions. Furthermore, the co-presence of identical and sub-identical clones of *tsa56* gene in geographically distant areas implies potential spread of *O. tsutsugamushi* genotypes. Fluctuation and diversification of vector species harboring *O. tsutsugamushi* in local endemic areas may facilitate genetic recombination among diverse genotypes. Therefore, careful monitoring of dominant vector species, as well as the prevalence of *O. tsutsugamushi* genotypes may be advisable to enable proper anticipation of epidemiological changes of scrub typhus.
Several Candidatus Rickettsia species have been proposed. Although the majority of these microorganisms still remain to be isolated, some of them have been implicated in human pathology (e.g. Ca. Rickettsia rioja). The identification of new genotypes and potentially new Rickettsia species is important for the management of arthropod-borne diseases. In a recent research, a Rickettsia amplified in ticks collected from donkeys in South Africa could not be identified to the species level. The ompA and ompB amplicons corresponding to one pool of 3 Rhipicephalus simus (obtained from 3 donkeys) showed 97.9% and 99.0% identity with those of a validated Rickettsia species. In the present study, the genetic characterization based on criteria agreed by experts was performed. DNAs from each half of the 3 ticks belonging to the positive pool were used as template for Rickettsia-specific PCR assays targeting ompA, ompB, 16S-rRNA, gltA and sca4 fragment genes. All samples tested positive and sequences for these genes showed the highest identities (97.8%, 99.1%, 99.3%, 99.8% and 98.8%, respectively) with the corresponding genes of R. africae as a validated Rickettsia species. According to the genetic criteria for Rickettsia identification, a new Candidatus status could be given. Nevertheless, the percentages of identity were closest to Candidatus Rickettsia barbariae (99.5%, 100%, 99.8%, 99.5% and 95%, respectively). More studies need to be conducted to determine the definitive status of this potentially new Rickettsia species found in R. simus in South Africa. Since R. simus bites humans, the potential pathogenic role of this microorganism should be considered.
Arthropod-borne *Rickettsia* species are obligate intracellular bacteria pathogenic for humans. Within this genus, *R. slovaca* and *R. conorii* cause frequent and potentially severe infections, whereas *R. raoultii* and *R. massiliae* cause rare and milder infections. All four species belong to spotted fever group (SFG) rickettsiae. However, *R. slovaca* and *R. raoultii* cause Scalp Eschar and Neck LymphAdenopathy (SENLAT) and are mainly associated to Dermacentor ticks whereas the other two species cause Mediterranean spotted fever (MSF) and are mainly transmitted by *Rhipicephalus* ticks. To identify the potential genes or biomarkers and to understand the evolutionary processes to that could, comprehensively, relate to the differences in virulence and pathogenicity observed between these four species, we compared their genomes and proteomes. The virulent and milder agents displayed a divergent phylogenic evolution into two major clades, whereas either SENLAT or MSF disease suggests a discrete convergent evolution of one virulent and one milder agents despite their distant genetic relatedness. Moreover, the two virulent species underwent strong reductive genomic evolution and protein structural variations, as well as a probable loss of plasmid(s), compared to the two milder species. However, an abundance of genes of the mobilome was observed only in the less pathogenic species. After infecting *X. laevis*, the virulent agents displayed less up-regulated than down-regulated proteins coded mainly by the core genome, as compared with the two milder agents. Furthermore, the virulent agents exhibited similar and distinct protein profiles that did not include some genes (*e.g.*, *ompA/B* and *rickA*) known to be related to rickettsial adhesion, motility and/or virulence, but may contain putative virulence-/antivirulence- and/or disease-related proteins. Overall, current SFG genomes have been shaped by several evolutionary processes suggesting a potential association of these driving forces with the emergence of distinct virulence and genomic plasticity between the four pathogens. These events may have a strong impact on intracellular protein expression and strategies in these rickettsiae, and that may lead to distinct virulence/antivirulence and diseases in humans. Thus, the current multi-omics data provide new insights into the evolution and fitness of the SFG virulence and pathogenicity, and the intracellular pathogenic bacteria.
Population of the common bed bug, *Cimex lectularius*, have recently undergone explosive growth, with the rapid increase of human infestation worldwide. Health consequences include nuisance biting and cutaneous and systemic reactions, as well as the potential for bed bugs to serve as disease vectors. *Cimex lectularius* are known to harbor the primary symbiont "Wolbachia" endosymbiont of *C. lectularius*. Endosymbiotic bacteria represent a promising method for pest control and eradication as they may induce a drastic reduction in arthropod fecundity and egg viability. Here we show that *Cimex lectularius* from laboratory maintained colony harbor another endosymbiont belonging to the *Rickettsiaceae* family.

Eight *Cimex lectularius* bedbug originating from laboratory colony were rinsed in distilled water and thoroughly sterilized with respectively by a solution of 10% of sodium hypochlorite followed by iodized alcohol. Each *Cimex lectularius* was transferred in individual 2 ml tube containing 400 µl of Schneider Insect medium and triturated by sterile glass pestles. 200µl of each suspension was inoculated in shell vials tubes containing a layer of an uninfected *Drosophila melanogaster* cell line (S2) grown in Schneider’s Drosophila Medium supplemented with 5% heat-inactivated fetal bovine serum and 1% of L-glutamine. Bacterial isolation was identified in 6 weeks by optical microscopy followed by sequencing the 16S RNA genes. Subculture on the *Xenopus laevis* cell line (XTC2) was conducted and growth of bacteria was observed after 15 days of subculture. The complete 16S RNA gene sequences exhibited 100% of identity with rickettsia identified in green rice leafhopper *Nephotettix cincticeps* (AB702995) and 99% with multiple *Rickettsia*-like endosymbionts including endosymbionts of the water beetles of genus *Deronectes* and the leech *Hemiclepsis marginata*. Phylogenetic analysis of the complete 16S RNA gene sequences revealed that Rickettsia-like from *Cimex lectularius* belongs neither to the ‘spotted fever group’ nor the ‘typhus group’. This is the first strain from the group representing leach- and insect-associated *Rickettsia*-like organisms. Its isolated phylogenetic position may mean that this group should be attributed to a new genus inside the *Rickettsiaceae* family.
Vector-borne diseases (VBDs) represent a major public health concern, and they account for more than 17% of the estimated burden of all infectious diseases affecting humans. The collection, identification, and molecular detection of *Rickettsia* and other bacteria carried by ectoparasitic arthropods removed from Algerian patient are discussed in this study. Most of the arthropods sent to our laboratory or collected by our team are those that reside for a length of time on or near the human host for most or part of their life cycle, such as ticks, lice, fleas, mites, and bed bugs. Thus, after identification of ectoparasite species, DNA samples of these specimens were screened by quantitative real-time PCR (qPCR) for *Rickettsia* spp. and other bacteria such as *Bartonella* spp. and *Borrelia* spp. Positive samples at the genus level were confirmed by qPCR specific species and/or by amplification and sequencing. Several pathogens or suspected pathogens of *Rickettsia* and *Bartonella* species have been identified among our specimens: *Rickettsia massiliae*, *R. slovaca*, *R. felis*, *R. felis*-like (genotype RF2125), and *Bartonella quintana*. Finally, other arthropods not of public health concern were identified and may be nuisance pests (Psocids "Booklice") and cause dermatologic manifestations by incidental contact (*Sclerodermus domesticus*).
The spotted fever group (SFG) rickettsiae are intracellular bacteria usually associated with ixodid ticks. Birds are hosts of many tick species and may contribute to the dissemination of ticks and tick-borne pathogens throughout the world. However there is still a lack of data on the occurrence of ticks on birds and associated rickettsial pathogens in Baltic countries. A total of 146 birds of 15 species infested with ticks were collected at Ventes Ragas ornithological station, Lithuania during 2013 and 2014. The ticks were identified as *Ixodes ricinus* using standard morphological and molecular techniques. A total of 284 ticks (109 larvae and 175 nymphs) were examined for the presence of the SFG rickettsiae using PCR amplification and sequence analysis of *gltA* and *17kDa* genes. *Rickettsia* DNA was detected in 10.2 % of the tick samples. Infected ticks were found on nine bird species *Erithacus rubecula*, *Troglydtes troglodytes*, *Sylvia communis*, *Coccothraustes coccothraustes*, *Luscinia luscinia*, *Parus major*, *Phylloscopus collybita*, *Regulus regulus*, *Prunella modularis*. *E. rubecula* were the predominant bird species infested by infected ticks. Sequence analysis of tested samples reveals presence of human pathogenic *R. helvetica* and *R. monacensis* with 98-100 % sequence similarity. This study is the first report of *Rickettsia* species in ticks from migrating passerine birds in Lithuania.
Rickettsiae are obligately intracellular bacteria with complex life cycles that are transmitted to vertebrates by a variety of arthropod vectors. Although in Europe some studies showed the occurrence of different Rickettsia species in their arthropod vectors, but only few studies have been conducted to identify reservoir hosts for these pathogens. The aim of the present study was to investigate the presence of Rickettsia spp. in different species of small rodents and to assess the potential role of these small mammals in the maintenance of Rickettsia spp. in Lithuania. A total of 334 small rodents belonged to the six species Apodemus flavicollis (n=175), Myodes glareolus (n=111), Micromys minutus (n=37), Microtus oeconomus (n=8), M. agrarius (n=2), and M. arvalis (n=1) were captured with live-traps in different locations in Lithuania during 2013–2014. Spleen samples of small rodents were examined for the presence of Rickettsia DNA by use of PCR targeting partial gltA and 17kDa genes. Rickettsiae were detected in 114 (33.9 %) of small rodents. In total, 37.7 % (66/175) of A. flavicollis, 27.9 % (31/111) of M. glareolus and 45.9 % (17/37) of M. minutus were positive. The sequence analysis shows that Rickettsia isolates from small rodents were 99-100% similar to Rickettsia helvetica sequences deposited in GenBank. This is the first report of Rickettsia helvetica found in small rodents in Lithuania. Our study suggests that rodents may act as reservoir hosts for Rickettsia pathogens.
It has been suggested that Coxiella burnetii, the causative agent of Q fever, is associated with non-Hodgkin lymphoma (NHL). We studied the association between chronic infection with C. burnetii and NHL in the Netherlands, by comparing incidence rates (IR) of NHL in chronic Q fever patients to the overall Dutch population based on data from the Dutch national chronic Q fever database and the Dutch integral cancer centre (IKNL). An adjusted relative risk for NHL was calculated using a negative binomial regression model. Of 439 chronic Q fever patients (mean age 65 years, male sex 73%), 5 developed NHL (absolute risk 1.1%, IR 3.01/1000 personyears). Three B-cell chronic lymphocytic leukaemias (non-hairy cell), one mantle cell lymphoma and one inconclusive subtype of B-cell NHL were observed. Median time between diagnoses chronic Q fever and NHL was 0.7 years (IQR 0.4 – 2.2). The relative risk adjusted for age and sex was 4.42 (95% CI 1.31 – 14.95). We conclude that incidence of NHL among chronic Q fever is increased compared to the general Dutch population. Causality and temporal relationship remains uncertain: both diseases have a considerably diagnostic delay and common risk factors such as immunocompromised state (although no NHL patients with chronic Q fever in this study had such risk factors). Finally, there is a risk of detection bias: regular monitoring of chronic Q fever patients may result in earlier diagnosis of NHL. Despite these drawbacks, clinicians should be aware of the increased incidence of NHL among chronic Q fever patients.
Coxiella burnetii antibody Seropositivity is not a risk factor for AIDS-related non-Hodgkin lymphoma

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Coxiella burnetii is the causative agent of Q fever, a disease that can lead to life threatening endocarditis and other serious conditions. About 3% of the US population has antibody evidence of exposure to this agent. Recently, the incidence of non-Hodgkin B-cell lymphoma (NHL) was found to be higher in French patients with C. burnetii infection relative to the general population. Individuals infected with human immunodeficiency virus (HIV) are predisposed to developing lymphomas including NHL and there is some evidence to suggest that HIV-positive individuals may be more likely to have anti-C. burnetii antibodies. Therefore, we hypothesized that anti-C. burnetii seroprevalence would be higher in individuals with acquired immune deficiency syndrome (AIDS)-related NHL relative to the general HIV-positive population. In this nested case-control study, we utilized ELISA and IFA assays for anti-C. burnetii antibodies to test 68 individuals with AIDS-related NHL and 67 HIV-positive controls from two US prospective AIDS cohorts. Overall, 11 (8%) of these HIV-infected individuals had anti-C. burnetii antibodies but seropositivity was not significantly different between NHL cases and controls. These findings suggest that C. burnetii infection is not associated with AIDS-related NHL.
A Q fever outbreak due to *Coxiella burnetii* genotype 17 occurred in a small military residential area in Cayenne, French Guiana. Retrospective cohort study was conducted to identify Q fever risk factors during this outbreak. Confirmed Q fever case was defined as positive serology (IgM ≥ 50 and phase II IgG ≥ 200) and/or positive qPCR on serum or blood. In addition, wild mammals were captured at the study site and tested by serology and real-time PCR performed on blood, vaginal swabs and ticks. The attack rate was 20 percent (11/54). All the cases were symptomatic with fever > 38.5°C and community-acquired pneumonia for four cases. Log binomial multivariate models identified two independent risk factors associated with Q fever: to clean the house (RRa=7.5 CI95% [1.03-55.3]) and to carry a three-toed sloth in arms (RRa=2.6 CI95% [1.1-5.8]). Eighteen marsupial individuals were captured, all PCR were negative but 17% (3/18) had a positive serology. Human contamination probably occurred through inhalation of infectious aerosols and through direct contact with three-toed sloth. Another study conducted after the outbreak among sheep, goats, wild mammals and birds living on and around the site found only one (1/4) three-tooth sloth (*Bradypus tridactylus*) with feces highly infectious for *C. burnetii* MST17 and 88% of his ticks were positives for *C. burnetii* using specific PCR. The same strain *C. burnetii* genotype 17 has been laboratory-confirmed in this mammal and in human cases. These results support the implication of three-toed-sloth in this outbreak. Human contamination probably occurs through inhalation of infectious aerosols, probably aerosols of dusts as suggested by high relative risk estimated with house cleaning activities and pulmonary forms of the disease, and through direct contact with three-toed-sloth. Positive serological results among marsupials confirm wildlife exposure and suggest a more complex sylvatic transmission cycle among wild mammals.
Coxiella burnetii endocarditis is one of the most serious Q fever complications, and its diagnosis is still challenging. Although antiphospholipid antibody syndrome with valvular vegetation in acute Q fever has been recently described as a new clinical entity, there are currently no established criteria for acute Q fever endocarditis. We proposed diagnostic criteria for acute Q fever endocarditis and a description of this new clinical entity based on the observation of a series of cases. In the French National Reference center (NRC) for Q fever, when positive serology is consistent with a C. burnetii infection, transthoracic cardiac echography is systematically requested to identify valvular lesions and to prevent and treat endocarditis. Clinical and imaging data are collected by phone. Acute Q fever endocarditis was definite when vegetation, nodule or valvular cordae tendinae rupture was observed in addition to one microbiological criterium (positive C. burnetii serology, PCR or culture). Acute Q fever endocarditis was possible when the valve was thickened, remodeled, calcified or when the cordae tendinae was thickened. Among 2'580 patient with positive C. burnetii serology, 1'730 had acute Q fever and 53 presented an acute Q fever endocarditis. Forty-two patients were men (79%) and mean age was 57 ± 13 year-old. Eighteen patients had definite acute Q fever endocarditis and 35 patients had possible endocarditis. Positive anticardiolipin antibodies (>22GPLU) was an independent predictor for acute Q fever endocarditis (OR=2.1, 95 confidence interval [1.1-4.4], p=0.02), thrombosis was associated with acute Q fever endocarditis (OR=6.4, 95 confidence interval [1.6-24], p=0.007) and acute Q fever endocarditis was a predictive factor for evolution to persistent C. burnetii endocarditis (OR=6.1, 95 confidence interval [3.2-11.5], p<0.001). Acute Q fever endocarditis confirm the tropism of C. burnetii for heart valves making Q fever a serious disease for which cardiac transthoracic echography is an indispensable diagnosis tool and anticardiolipin antibody a predictive biomarker.
Q fever could be responsible of 25% of hospitalized lobar pneumonia in endemic area. Pulmonary fibrosis in the aftermath of C. burnetii infection has been questioned for decades, but few cases of interstitial lung disease (ILD) in association with Q fever have been reported. We diagnosed a case C. burnetii persistent infection in a patient with ILD with histological lung fibrosis, and we identified C. burnetii in situ by immunohistochemistry and Fluorescent in situ Hybridization (FISH). This index case prompted us to investigate the link between ILD and Q fever. In the French National Reference center (NRC) for Q fever, when positive serology is consistent with a C. burnetii infection, clinical data are collected by phone to determine the infective focus and to adapt therapeutic management. ILD was diagnosed by a multidisciplinary team, after a CT-scan centralized reviewing that follows the international recommendations (American Thoracic Society and the European Respiratory, 2013). Over 25 years experience of the NRC for Q fever, we screened 2'580 patients with C. burnetii positive serology consistent with Q fever among which 534 (20%) presented pneumonia. Seven patients presented ILD in a context of C. burnetii infection. Mean age was 67.5 ± 11 year-old and men were 5. Two patients presented acute Q fever and 6 had persistent C. burnetii infection among which 4 were associated with another focus (3 persistent endocarditis and 1 granulomatous hepatitis). CT-scan imaging showed usual interstitial lung disease and specific interstitial lung disease for one patient respectively, lesions were unclassifiable for 5 patients who presented severe lesions of interstitial lung fibrosis. Four patients were hospitalized in intensive care unit and one of them died. The disease stabilized for other patients. Based on C. burnetii specific immunohistochemistry, polymerase chain reaction, FISH, serology and CT scan imaging, we proposed new criteria to diagnose ILD as a C. burnetii infective focus. Interstitial lung disease is a rare, serious and new clinical manifestation of Q fever, which must be considered with interest.
The closely related species *Rickettsia conorii* and *R. africae* are both etiological agents of rickettsiosis, a tick-borne serious infective disease. The laboratory diagnosis is mainly based on serological methods. However, this technique requires the production of rickettsial antigens, which is burdensome. Moreover, whole cell serology remains not enough specific to provide the diagnosis at the species level. Here, we attempted to identify specific proteins that would enable the discrimination of *R. africae* sp from *R. conorii* sp infections. We screened 22 *R. africae*- and 24 *R. conorii*-infected sera at different course of infection using a traditional immunoproteomic approach. In parallel, we focused on the technical development of a “relatively new technique” named a proximity ligation assay coupled to two-dimensional Western blotting (PLA WB). We proposed several proteins discriminating these two rickettsial species at early and active stages of infection. The top range markers of *R. africae* early infection were rpoA, atpD, and acnA, *R. africae* active infection were rOmpB β-peptide, OmpA, groEL and ORF1174, early *R. conorii* infection was prsA, *R. conorii* active infection were ftsZ and cycM. Our results suggest that top range markers are most likely candidate antigens for serodiagnosis of rickettsioses.
Q fever is a zoonotic disease caused by Coxiella burnetii. It is considered that farm animals, pets and ticks are the main reservoirs of infection and the transmission to human beings is accomplished through direct contact or inhalation of contaminated aerosols. In Korea recently C. burnetii is tend to increase but there is limited genetic information of Q fever human cases. In this study we investigated the genetic feature of two Q fever patient to understand where their C. burnetii are included.

Suspected patient’s buffycoat were inoculated into immune-depressed Balb/c mice via the intraperitoneal route for amplifying C. burnetii existing in patient’s blood. After that we got lung, liver, spleen of the mice. Extracted DNA of those tissue were analyzed using 16S rRNA and IS1111 of C. burnetii.

After inoculating buffycoat into mice, enlargement of mice spleen were shown. The result of amplification of C. burnetii specific 16S rRNA and IS1111 revealed that infectious disease of those patient was Q fever. Phylogenetic analysis showed it had a sequence similarity (90.04-97.56%) with C. burnetii 16S rRNA and (94.69%-100%) with C. burnetii IS1111 sequences in one patient. In the other patient’s result sequence similarity was 99.6-100% with C. burnetii 16S rRNA and 94.69%-100% with C. burnetii IS1111 sequences.

This experiment using animal amplification was useful to diagnose as a Q fever and infer their origins although it was very laboritious. For further studies, pathogenicity or full genome sequencing will be needed to understand the characteristics of Korean C. burnetii isolates.

Acknowledgements: This work was supported by Korea Centers for Disease Control and Prevention (4838-303-210-13)
An association between infection with Coxiella burnetii and non-Hodgkin lymphoma has been suggested. To further explore the pathogenesis, we assessed presence of C. burnetii in B-cell NHL tissues (BNHL) of patients previously exposed to C. burnetii. We used C. burnetii specific polymerase-chain reaction (PCR), RNA-fluorescence in-situ hybridization (RNA-FISH) and immunofluorescence (IF). Patients were selected from an area in the Netherlands were Q fever was highly endemic in 2007–2010. Previous exposure to C. burnetii was defined as phase II IgG antibody titers ≥1:32, persistent infection as phase I IgG antibody titers ≥1:1024. Of 136 patients with BNHL, 18 (13%) had been previously exposed to C. burnetii, of which 2/18 (11%) had persistent infection. Of 18 patients, 14 BNHL samples were available (6 lymphnodes, 2 bonemarrow, 1 stomach, 1 coecum, 1 spleen, 1 lung, 1 retroperitoneum, 1 nasopharynx). C. burnetii PCR performed on these tissues were all negative. However, both RNA-FISH and IF demonstrated C. burnetii in 5/14 cases (36%): in 1 patient with persistent vascular infection (diagnosed simultaneously with BNHL) and 4 patients with past infection. Mean time between positive serology and development of BNHL in patients with past infection was 3.3 years (range 0.02–6.70). The results of this study suggest a relation between C. burnetii infection and NHL. The diagnostic value of PCR seems limited, compared to FISH and IF. The implication of presence of C. burnetii in (malignant) tissues requires further research: additional evaluation of presence of C. burnetii in non-malignant tissues will follow.
Arterial fistulae have been described as complication of vascular chronic Q fever. If left untreated, they are fatal. We performed a retrospective, observational study to assess the cumulative incidence and mortality of arterial fistulae during chronic Q fever. Data of patients with proven chronic Q fever were extracted from the national Dutch chronic Q fever database (probable and possible chronic Q fever patients were excluded since definite diagnosis is not certain in these patients). Of 253 patients with proven chronic Q fever, 127 (50%) patients had a vascular focus, 42 (17%) patients had a combined vascular focus and endocarditis. Among 169 patients with a vascular focus of infection (alone or combined), 25 (15%) patients developed 26 arterial fistulae: 15 aortoenteric, 2 aortobronchial, 4 aortocaval and 5 arteriocutaneous fistulae (1 patient with both aortovenous and arteriocutaneous fistula). Primary fistulae accounted for 42% and secondary fistulae for 58%. Chronic Q fever-related mortality was 60% for patients with arterial fistula and 21% for patients without arterial fistula (p<0.0001). Both patients with aortobronchial fistulae (n=2) and 80% of patients (n=12) with aortoenteric fistulae died of chronic Q fever. We conclude that the proportion of patients with chronic Q fever developing primary and secondary arterial fistulae is high. Furthermore, chronic Q fever-related mortality is high in these patients. Clinicians should be alert on signs and symptoms matching arterial fistula in areas were Q fever is or has been endemic and should consider the diagnosis Q fever in patients presenting with arterial fistula.
Coxiella burnetii, the causative agent of Q fever, causes abortion in small ruminants and infected animals shed the bacteria at lambing contaminating the farm environment, where it can survive during long periods. Contaminated aerosols are the main source for human infection. To study the evolution of Coxiella burnetii infection in four naturally-infected sheep flocks where no specific control measures had been implemented, vaginal swabs, milk and faeces from a maximum of 40 ewes and 40 yearlings per flock as well as dust samples were taken within one week after lambing during two consecutive lambing seasons (2015/16 and 2016/17). Aerosols were also collected outdoors and indoors after lambing at monthly intervals. Analysis of all samples by Real-Time PCR targeting the IS1111 gen showed different patterns of shedding, indicating that Q fever was introduced in each flock at different times. During the first lambing season, an average of 66.3% ewes (120/181) and 60.5% yearlings (69/115) shed Coxiella through at least one of the excretion routes. The following lambing season a significant decrease in ewes shedding was observed (10.0%, 16/160). Independently of the percentage of shedders, all farms presented positive aerosols. Most of the dust samples were C. burnetii-positive but a progressive decrease in bacterial load was observed. Significant correlations between percentage of animal shedders and bacterial burden in dust and indoors aerosols were found. Studies on C. burnetii viability in environmental samples are in progress to determine how long C. burnetii remains viable in farms with a previous episode of Q fever.

Acknowledgements: Funded by INIA RTA2013-00051-C02-01
Infection with *Coxiella burnetii* in cattle is in most cases sub-clinical or asymptomatic unless in specific conditions where it might cause metritis, mastitis, infertility and, in pregnant animals, abortion. This study aimed at characterizing, by the use of a double genotyping approach (SNP and 13-locus MLVA analyses), *C.burnetii* strains linked with symptomatic infections in cattle, namely during abortion. Between the years 2010 and 2016, the animal Belgian National Reference Laboratory served as first line screening for Q fever within the national abortion protocol campaign. Roughly 10.000 diagnostic PCR tests were run yearly in aborted material, more than 90% involving bovine samples (placentas, abomasum fluids, foetuses). To investigate the variability of *C. burnetii* strains directly related to abortion in cattle, only highly loaded samples (Ct<20) were further selected for typing analyses. SNP typing (Dutch scheme) highlighted the high monogenetic phylogeny of the strains, with the SNP type 2 present in 98.6% of cases. Within this genotype, MLVA was slightly more discriminatory with the MS24 and MS34 providing the level of variability. One sample had the CbNL01-like genotype, the genotype circulating in Belgian strains and having the same MLVA type as the strain causing the 2007-2010 outbreaks in the Netherlands. This phenomenon related to a cross species transmission from caprine strains. Overall, results of this study confirmed the homogenic pattern for *C.burnetii* strains associated with abortion in cattle and highlight on the usefulness of screening and typing of abortions for surveillance of spill-over of epidemic strains.
The aim was investigation of C. burnetii genetic heterogeneity for understanding its pathoadaptation to host species diversity using the strains of the Russian population. We performed whole genome sequencing of 4 C. burnetii strains isolated from two host types (the human and the arthropods) using the MiSeq technology with the paired-end and bar code strategies, according to the manufacturer’s instructions (Illumina, USA). Genome assembling and alignment using Dugway 5J108-111 as the reference genome was performed with SPAdes 3.9.0 genome assembler and it was annotated using MyRAST. We used IGV browser for assembling estimation and Mauve 2.4.0 tool for genomes alignment and comparison.

Analysis of 4 whole genomes was performed both within the group of Russian strains and with genomic comparisons using 9 C. burnetii complete genomes that are available from NCBI and showed pronounced clustering within a group of Russian strains by host type, the differences between genomes within clusters were minor. Comparing the number of deleted ORFs for Russian strains, it was found that surprisingly strains from arthropods had a significantly greater reduction of the genome compared with strains from human, and they demonstrated the greatest similarity in genomotype with the genomotype of the Cb175 Guyana strain isolated from the human. Thus, despite the obvious clustering of Russian strains by type of host, the data suggest ambiguity about the origin of the strain from a particular macroorganism on the basis of data on the deletion of certain genes from its genome and may be of a presumed character.
The *Coxiella burnetii* strain NL3262 was isolated during the Q-fever outbreak in the Netherlands in 2007-2010 [Kuley et al., 2016]. Formal Order Analysis (FOA) [Gumenuk et al., 2013] was used to study the similarity of the genome (chromosome and plasmid) of this strain with the genomes of other strains. All genomes have been imported from the database GenBank NCBI (USA): www.ncbi.nlm.nih.gov/genome. Chromosomes of 8 strains *C. burnetii* (Dugway 5J108-111; CbuK_Q154; Z3055; RSA 493; CbuG_Q212; RSA 331; NL3262 and «MSU Goat Q177») and 6 plasmids (Dugway 5J108-111 pQpDG; CbuK_Q154 pQpRS; RSA 493 pQpH1; RSA 331 QpH1; NL3262 QpH1 and «MSU Goat Q177» pQpRS) were studied using the tools «Map of genes» (MG) [Pozdnichenko et al., 2016] and «Matrix of similarity» (MS) [Gumenuk et al., in press]. MG showed that the chromosome of strain *C. burnetii* str. NL3262 by index of average remoteness (g) - 1.448640 (X axis) distanced itself from chromosomes (g 1.448295 - 1.448865) other strains. 106 copies of «IS110 family transposase» were detected in the chromosome of str. NL3262, in other strains their number varied from 0 to 44. Analysis of components (coding and non-coding) of chromosomes ranked according to the depth index - G (Y axis) showed that more than 80% of the components of *C. burnetii* Z3055 and NL3262 strains had complete coincidence of nucleotides (100% homology). The MS was used for an advanced analysis of the results obtained. The complete similarity of the components of chromosomes and plasmids was determined by pairwise comparison and the identification of nucleotides coinciding with them. 86.57% of the components of the chromosome of *C. burnetii* str. NL3262 coincide completely with the components of the chromosome of str. Z3055, for chromosomes of other strains this index was from 12.06% to 48.37%. The plasmids were present in six strains of *C. burnetii* only. The plasmids (QpH1) of the RSA 493, NL 3262 and RSA 331 strains formed a compact group, remote from the other plasmids by g index. The plasmid of strain NL3262 had 50.0% of the components with complete coincidence with the components of the plasmid RSA 331, with RSA 493 - 31.17%, for the other strains from 5.77% to 6.9%. Thus, *C. burnetii* str. NL3262 is the closest to the str. Z3055 by similarity of the components of chromosomes, and it plasmid QpH1 to the str. RSA 331.
Coxiella burnetii is an obligate intracellular pathogen and the causative agent of the zoonotic disease Q fever. Upon uptake by alveolar macrophages the pathogen is able to replicate in an acidic phagolysosomal vacuole (PV). For the establishment of the PV effector proteins translocated into the host cell by the type IV secretion system (T4SS) are important. Here, we are focusing on the effector protein AnkF and its role in the establishment of the PV. The C. burnetii AnkF transposon mutant infects host cells as efficient as wildtype C. burnetii. However the AnkF mutant is unable to replicate intracellular, indicating that AnkF might be involved in the establishment of a replicative PV. This hypothesis was supported by the fact that Legionella pneumophila translocating AnkF into the host cell is defective in establishing a replicative L. pneumophila-containing vacuole. To determine the underlying reason we searched for host cell binding proteins by yeast two-hybrid and identified Vimentin. While AnkF does not alter Vimentin on mRNA or protein level, expression of AnkF results in structural reorganization and vesicular co-localization with recombinant Vimentin. Ectopically expressed AnkF partially accumulates around the established PV and endogenous Vimentin is recruited to the PV in a time dependent manner, suggesting that AnkF might attract Vimentin to the PV. However, siRNA-mediated knock-down of Vimentin indicates that Vimentin is dispensable for intracellular replication of C. burnetii. Taken together, AnkF is essential for the establishment of the replicative PV, however, its mode of action is so far elusive.
Q fever is a zoonosis caused by the *Coxiella burnetii* bacterium. Domestic ruminants are the major reservoirs of the disease and their main clinical manifestations are abortions. To date, the method, most commonly used for the diagnosis of Q fever abortions is a quantitative polymerase chain reaction (qPCR) assay based on the amplification of the multicopy IS1111 transposon-like element. This method is highly sensitive and rapid to perform. However, because the number of IS1111 elements is highly variable between strains, the estimation of bacterial burdens shed by infected females may be biased. Here, we aimed to determine the number of IS1111 elements in various *Coxiella burnetii* strains from animals prevailing in France, in reference to a monocopy gene. Analyses were carried out on DNA samples from vaginal swabs of Q fever aborted females between 2006 and 2015. Two plasmid calibration standards were constructed, containing either the multicopy IS1111 or the monocopy icd target. Separate TaqMan-based qPCR assays were used to quantify both targets in each sample.

Significant differences between samples, regarding the number of copies of the IS1111 elements, were observed depending on the ruminant species: strains from sheep displayed a higher number than goat’s one, which displayed a higher number than strains from cattle. Moreover, these results suggest that the estimation of bacterial burdens shed by infected females is slightly overestimated, especially in small ruminants, when the multicopy IS1111 element is used as a qPCR target. However, the use of a 20 copy average number, as suggested by the OIE Q fever laboratory (ANSES Sophia Antipolis, France), is suited for the diagnosis of Q fever abortions. Overall, this study highlights the need to use a standard range based on a well characterized reference material to avoid quantification biases in routine laboratory analysis.
There is currently no vaccine licensed in the UK or USA for prophylaxis against the bacterial biothreat agent *Coxiella burnetii*, which causes the disease Q fever. Isolates of *C. burnetii* exist in two phases that differ in lipopolysaccharide (LPS) production and virulence. Phase I variants produce a smooth LPS characterised by a full length O-antigen. A phase I formalin inactivated whole cell vaccine, Q-Vax, is reported to be highly protective but is only licensed for use in Australia. It requires pre-screening prior to administration and severe localised and systemic reactogenicity has been reported in vaccinees. Phase II bacteria produce a rough, truncated O-antigen and are not protective as a killed vaccine. This indicates that LPS is a key protective antigen and should be included in any sub-unit vaccine to be produced. Several protein antigens have been tested as potential vaccine candidates with poor success. However, it is likely that promotion of a specific T-cell response will be required of any protective Q fever vaccine, therefore the inclusion of protein antigens in the subunit vaccine may be pivotal.

The production of native LPS from *C. burnetii* is technically challenging and requires cultivation of large bacterial numbers in a high containment laboratory. However, this material has been used to demonstrate efficacy in our established inhalational mouse model of Q fever. In order to produce a more defined, scalable vaccine candidate, an alternative technical approach of producing synthetic saccharide fragments is being investigated. Polysaccharides are considered T-cell independent antigens, inducing poor levels of immunological memory and previous studies have demonstrated that conjugation of polysaccharides to a protein carrier can be a method of establishing a more appropriate T-cell dependent immune response.

Recombinant virus-like particles (rVLP) are non-infectious but highly immunogenic protein particles and are thus a vaccine vector of choice for less potent antigens. Mologic have developed a highly immunogenic rVLP which is designed to carry foreign antigens (or for site specific conjugation of antigens). Target proteins and polysaccharide antigens will be presented in a highly immunogenic context, stimulating strong and appropriate T- and B-cell responses. Therefore, to investigate improving the efficacy and developing memory to the *C. burnetii* O-antigen, a VLP vaccine candidate will be developed and tested for efficacy in an inhalational mouse model of Q fever.
In one to five percent of all acute Q fever infections, chronic Q fever develops, mostly manifesting as endocarditis, infected aneurysms, or infected vascular prostheses. In this study, we investigated the diagnostic value of FDG-PET/CT in chronic Q fever at diagnosis and during follow-up. All Dutch adult patients suspected of chronic Q fever who were detected since the start of the Dutch Q fever epidemic in 2007 were retrospectively included until March 2015 when at least one FDG-PET/CT was performed. Clinical data and results from FDG-PET/CT scans at diagnosis and during follow-up were collected. 273 patients suspected of chronic Q fever were included. Of all 230 FDG-PET/CT scans performed at diagnosis, 31 scans (13.5%) led to a change in diagnosis. FDG-PET/CT showed infectious foci in 63.3% of all 147 patients with proven chronic Q fever. At diagnosis, 45 scans (19.6%) led to treatment adjustment. Of all 218 scans performed during follow-up in 143 patients, two scans (0.9%) led to a change in diagnosis and 125 FDG-PET/CT scans (57.3%) resulted in treatment adjustment. When adding FDG-PET/CT to the Duke criteria as major criterion, 17 patients (1.9-fold increase) had a definite endocarditis. Q fever-related mortality rate in patients with and without vascular infection based on FDG-PET/CT was 23.8% and 2.1%, respectively (p= 0.001). FDG-PET/CT is a valuable diagnostic technique in localizing chronic Q fever and during follow-up often leading to a change in diagnosis and/or adjustment of treatment.
P3-29) Basma El Hamzaoui – Detection of Bartonella Quintana in fleas by MALDI-TOF MS

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Background
Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has recently emerged in the field of entomology as a promising method for the identification of arthropods and the detection of associated pathogens.

Methodology/Principal Findings
An experimental model of Ctenocephalides felis fleas (cat fleas) infected by Bartonella quintana was developed to evaluate the efficiency of MALDI-TOF MS to distinguish infected from uninfected fleas. Two groups of fleas received three successive blood meals infected or not by B. quintana. A total of 77 and 22 fleas engorged on human blood infected or uninfected with B. quintana, respectively, were collected. Fleas were dissected longitudinally; one half was used for assessment of B. quintana infectious status by real-time PCR, and the second half were subjected to MALDI-TOF MS analysis. Comparison of MS spectra between infected fleas and uninfected fleas revealed distinct MS profiles. Blind tests against our MALDI-TOF MS arthropod database, upgraded with reference spectra of infected and non-infected fleas provided correct classification for 100% of the different categories of specimens tested. The MALDI-TOF MS differentiated successfully infected and uninfected fleas by B. quintana. MALDI-TOF MS correctly identified flea species as well as their infectious status, which was consistent with the results of real-time PCR.

Conclusions/Significance
MALDI-TOF is a promising tool for identification of the infection status of fleas infected by Bartonella spp., which allows new perspectives for a fast and accurate diagnosis in medical entomology and vector surveillance.
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology, a routine tool for microbial identification has now emerged as an innovative tool in the entomological field for arthropods identification. Recent and numerous studies have reported the reliability of this tool for the identification of fresh or alcohol preserved ticks based on the mass spectra obtained from the legs. The aim of this study was to evaluate the performance of MALDI-TOF MS for the identification of Algerian alcohol preserved ticks collected from different domestic and wild hosts. In a second part a molecular survey for the presence of bacteria have been done for all the ticks studied by MALDI-TOF MS. A total of 2635 Ixodid and 1401 Argasid ticks were collected from animals in nine different regions of the North Eastern of Algeria. The collection included 10 distinct species belonging to 5 genera. The legs of 219 specimens were subjected to MALDI-TOF MS assays. Spectral analysis revealed intra-species similarity and inter-species specificity of the generated MS spectra which was consistent with the morphological identification. Blind tests against the customized database revealed that all specimens were correctly identified. The morphological and MALDI-TOF MS identification were controlled by sequencing the 12S ribosomal RNA gene (rRNA) for 58 specimens. All the ticks were correctly identified but the sixteen specimens of *Rh. turanicus* were molecularly identified as *Rh. sanguineus*. The molecular tests done by qPCR, showed that from 219 tested ticks 15 were positive for *Rickettsia* spp., 8 for *Borrelia* spp. and 17 for *Anaplasmataceae*. The tests were negative for *Coxiella burnetii* and *Bartonella* spp.

This study indicates once more that MALDI-TOF MS is a reliable tool for the identification of arthropods and brings a new data shed light on the tick species-diversity and tick-borne diseases in Algeria.

**Keywords**: Ticks; MALDI-TOF; tick-borne diseases; Algeria.
The genomics era started in 1995 with the complete sequencing of the *Haemophilus influenzae* and *Mycoplasma genitalium* genomes. Whole genome sequencing soon appeared as a powerful method to reveal bacterial properties, especially those of fastidious microorganisms such as intracellular bacteria. Moreover, comparative genome analysis of a large number of related strains have recently emerged as a cost-effective and convenient approach for addressing many microbiological questions, such as evolution, outbreaks, antibiotic resistance, and pathogenicity.

Herein, we used genomic data to describe the new rickettsial species *Rickettsia gravesii*. Considering its strictly intracellular lifestyle and the few expressed phenotypic characters, traditional identification methods used in bacteriology are inapplicable. For this aim, we used genomic comparison, the Average Genomic Identity of orthologous gene Sequences (AGIOS), COG categories and the digital DNA-DNA hybridization (dDDH) value. In a parallel ongoing study, genomic analysis was applied to 76 *Coxiella burnetii* genomes in order to understand the genetic diversity, pathophysiology, phylogeny and metabolism of this intracellular human pathogen.
Canine monocytic ehrlichiosis is a tick-borne disease caused by an intracellular alpha-proteobacteria, *Ehrlichia canis*, which replicates within mononuclear cells in the host. The present study was designed to use a PCR protocol for the molecular detection of *E. canis* by the amplification of their 16S rRNA gene, as well as the effects of this alpha-proteobacterium on the blood chemistry of the sampled dogs, and the risks factors associated with *E. canis* infection. A total of 151 blood samples were collected from dogs of various breeds at three sampling sites (Lahore, Rawalpindi/Islamabad, and Multan) in Punjab, Pakistan. Data regarding the epidemiological factors (including age, gender, breed, body temperature, deworming, vaccination, mucus membrane status, hydration status, presence of hematuria) and tick infestation were collected through a questionnaire at the time of sample collection. After PCR amplification, the 400 bp DNA fragment specific for 16S rRNA gene of *E. canis* was amplified from 42 dog blood samples (28 % of the total), [Lahore (N = 24), Rawalpindi/Islamabad (N = 13) and Multan (N = 05)]. Data analysis revealed that the character of the animals (age, sex, and breed) had no significant association (p > 0.05) with the presence of *E. canis*. Various hematological parameters were also compared, and the results revealed that all of the parameters remained unaffected, except significantly increased white blood cell counts (p =0.004) in *E. canis* positive blood samples, as compared to the control group. We concluded that PCR is a reliable molecular technique for the detection of *E. canis* DNA in dogs, and the presence of *E. canis* affects the white blood cell count of infected dogs. Moreover, no specific epidemiological parameter was found associated with the prevalence of *E. canis* in dogs.
Type I Interferons (T1 IFNs) induced by viral infections play a pivotal role in protective immune responses. Recently, various bacterial pathogens have also been shown to cause T1 IFN production. However, the effect of T1 IFNs induced by bacterial infections remain elusive. *Orientia tsutsugamushi*, an obligate intracellular bacteria causing scrub typhus, can also induce T1 IFNs during mammalian infection. Here, we studied the molecular details involved in the induction of T1 IFNs during *O. tsutsugamushi* infection *in vitro* and *in vivo*. We also investigated potential role of T1 IFNs in generating adaptive immune responses such as *O. tsutsugamushi*-specific antibody and T cell responses using T1 IFN receptor (T1 IFNR) knockout (KO) mice. Even though there was no significant difference between wild type and T1 IFNR KO mice in their morbidity and mortality during *O. tsutsugamushi* infection, degree of antigen-specific T cell responses maintained higher in T1 IFNR KO mice than wild type. These results suggested that acute T1 IFN responses induced by *O. tsutsugamushi* might be associated with immunopathogenesis and/or immune memory.
P3-34) Q fever in cattle, sheep and goats: results of a 2012-2015 study in France

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Q fever is a widespread zoonosis caused by the bacterium *Coxiella burnetii*, isolated from a wide range of animals (mammals, birds and arthropods), while domestic ruminants (goats, sheep and cattle) are considered the most common animal reservoir for human infection. The epidemiological situation at the national scale regarding Q fever in ruminants is poorly known in France. The few available data originate from local and heterogeneous studies based on various sampling protocols and laboratory methods. Thus, a global approach was discussed within the framework of the French platform for animal health surveillance by collaborative multi-stakeholder groups. From 2012 to 2015, a large-scale study was carried out in ten départements of France to estimate the clinical incidence (frequency of abortions related to Q fever in herds experiencing abortive episodes) and serological prevalence of Q fever in cattle, sheep and goat herds. Clinical incidence at the herd level was estimated by investigating series of abortions in 2,695 cattle, 658 sheep and 105 goat herds based on qPCR and ELISA analyses. Additionally, a serological survey was conducted on randomly sampled herds considering 731 cattle, 522 sheep and 349 goat herds. Both clinical and serological surveys confirmed that Q fever is circulating in French cattle, sheep and goat farms. Strong variability was observed between départements and species, suggesting that herd density and farming practices play a major role in the disease epidemiology. Overall, these data constitute a first reference to better assess the significance of future Q fever emergences.
Rickettsia felis, the agent of flea-borne spotted fever rickettsiosis, is worldwide in fleas, as well as in ticks, mites and mosquito. More recently, the cosmopolitan psocid Liposcelis bostrychophila (booklice), non hematophagous arthropod, was also shown to harbor strain of R. felis which is almost genetically identical to flea-associated R. felis. Our laboratory, reported the presence of R. felis DNA in 100% of dust samples collected from the beds of R. felis-infected patients in Senegal. These dust samples contained live L. bostrychophila, so we hypothesized that these psocids may serve as a source of human infection with R. felis when inhaling these infected dusts.

To investigate whether R. felis-infected psocids is pathogen in vertebrates, SCID mice were inoculated with suspension of infected booklice using a "whole body aerosol system" and blood and organs were collected different time points post-inoculation (PI) to follow up the apparition of infection. Several tissues from aerosol challenged mice including blood (at 1, 2 and 6 days PI), lungs, liver and spleen (at 6 days PI) were found positive for R. felis DNA. We suppose that contamination resulted essentially from inhalation of contaminated aerosols; however we cannot exclude the involvement of an external source (normal co-grooming/social behavior) in this contamination.

These results indicated that infection of mice with R felis-infected psocids occurs when using aerosol route. Further experiments are needed to confirm these results as well as to clarify the exact mode of contamination.
## 50 YEARS OF INTERNATIONAL RICKETTSIOLOGY MEETINGS

1967-2017

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