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Meeting report

ESCCAR international congress on Rickettsiae and other intracellular bacteria

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The ESCCAR international congress on Rickettsia and other intracellular bacteria is a meeting jointly organized by the American Society of Rickettsiology and the European Society of *Chlamydia*, *Coxiella*, *Aanaplasma* and *Rickettsia* (ESCCAR). Noteworthy, the first rickettsial meeting was organized in Smolenice (Slovakia) already 50 years ago by N. Kordova in 1967 whereas the first ESCCAR meeting was organized in Marseille (France) in 2008 by Philippe Brouqui. The 2nd ESCCAR meeting was then held in Heraklion (Greece) The last ESCCAR meeting took place in Lausanne (Switzerland) two years ago, from 13th to 16th June 2015. PE Fournier and G Greub were the president and vice-president of ESCCAR during last two years and have been the major organizers of the present Marseille meeting.

The Marseille meeting held during the first week of June, was a great success, with 254 participants from 33 different countries, originating from all 5 continents. There was a total of 107 oral presentations including 13 invited talk on *Chlamydia* or chlamydia-related bacteria, 11 invited talk on *Rickettsia*, 22 invited talks on other intracellular bacteria and 61 oral presentation selected from abstracts. In addition, 102 posters were presented during three specific interactive poster sessions.

1. Scientific lectures

The ESCCAR-ASR meeting started by **Didier Raoult**'s keynote on rickettsial major discoveries in the past 3 decades. He nicely showed that hypothesis-based research is not the only way to broaden our knowledge and that technology-based research that allows to fish for new pathogens is also very important, despite being until recently not recognized by most funding agencies. Nevertheless, as exemplified by *Rickettsia felis* culture in frog cell lines, to recover a new species in culture may rely on hypothesis, i.e. that an arthropod-associated bacteria likely grows at a lower temperature (28 °C) than mammals associated-rickettsia. *R. felis* is also a nice example of genes exchange, this bacterium exhibiting a very high level of mosaicism, explaining its larger genome as compared to other rickettsial species. Finally, he showed that in research we have to expect the unexpected. As an example, *R. felis* is likely also transmitted by mosquito (and not only by cat fleas) and may cause in Africa a common skin eruption (called "yaaf") associated with fever and headaches [1].

1.1. *SESSION 1 and 6 ter:* Rickettsia epidemiology

In session 1, **Maureen Laroche** (France) described the use of Matrix-Assisted Laser Desorption/Ionization-Time Of Flight mass spectrometry (MALDI-TOF MS) for arthropod identification. Specific peptide extraction protocols were developed and a spectrum database implemented for arthropods. The arthropod legs are usually used for MALDI-TOF MS identification to preserve the rest of the insect body for culture of bacterial symbionts and DNA extraction. To date, MALDI-TOF MS is used to efficiently identify hard ticks, cat fleas and mosquitoes. In addition, MALDI-TOF MS spectra from *Rickettsia*-infected insects were added to the database, making this method an efficient, rapid and inexpensive method for human-biting athropods.

Then, **Christopher D. Paddock** (USA) made a nice overview of the current knowledge on rickettsioses in the USA, starting with the belief, at the turn of the third millennium, that only one of these infections, Rocky Mountain spotted fever (RMSF), was endemic in the country. However, in the past 15 years, another two rickettsioses were detected, including those caused by *R. parkeri* and *Rickettsia philipii* in the southeastern, southwestern and western states. In addition, the epidemiology of RMSF changed, the disease being responsible for outbreaks in impoverished populations of southwestern states were it is transmitted by *Rhipicephalus sanguineus* instead of *Dermacentor* ticks in other areas. Dr Paddock highlighted the need to keep

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surveying tick-borne rickettsioses whose epidemiology might change rapidly according to climate and/or demographic changes.

Four short presentations were also part of session 1. **Silke Wolfel** (Germany) reported the presence of *Rickettsia monacensis* in *Ixodes ricinus* ticks from northern Denmark. Using multi locus sequence typing, she detected the bacterium in 4.9% of 367 tested tick pools and found no difference between German and Danish strains. **Michelle Allerdice** (USA) delivered a talk on the prevalence of *R. parkeri* in *Amblyomma maculatum* ticks in southern Arizona. By studying 182 ticks collected at three locations in national parks, she detected the bacterium using 23S rRNA and *ompA* PCRs in 41 ticks. Depending on locations, the prevalence of the bacterium ranged from 21 to 40%, thus representing a risk for people visiting national parks. Then, **Dalyte Mordosaite-Busaitiene** (Lithuania) reported the results of a study on rodents in the Curonian Spit, a region of Lithuania located by the Baltic Sea. On the 238 captured rodents, a total of 1226 ectoparasites were collected. 16.3%, 7.9% and 43.5% of ticks, mites and fleas were positive for rickettsiae, respectively. *R. helvetica* was detected in ticks, mites and fleas, *R. felis* in mites and fleas and *R. monacensis* in fleas. Other uncharacterized species were also detected in mites. Finally, **Ilaria Pascucci** (Italy) detailed the results of a study aiming at evaluating the diversity of *Rickettsia* species in ticks in the Abruzzo and Molise regions of central Italy. A total of 603 ticks were collected and tested in 178 pools. Using 23S rRNA, *gltA* and *ompA* PCR, they found 93/178 pools (52%) to be positive. Eight *Rickettsia* species were identified, mainly *R. slovaca* and *R. aeschlimannii*, but also *R. conorii*, *R. felis*, *R. helvetica*, *R. massiliae*, *R. monacensis* and *R. raoultii*.

In SESSION 6 ter, **Kevin Macaluso** (USA) focused his talk on *R. felis* and its transmission by fleas. Then, **Emmanouil Angelakis** (France) presented the current knowledge on *Rickettsia sibirica mongolotimonae*, which represents one of the more common species in France, being more frequent than *R. conorii* and *R. slovacae*.

1.2. *SESSION 2:* updates in the Chlamydiales field

The first talk of this session was given by **Gilbert Greub** (Switzerland) and was focusing on the biology of chlamydia-related bacteria. This includes all members of the chlamydiae phylum that are not member of the Chlamydiaceae family. It thus encompasses 9 family-level lineages. In his talk, G. Greub specifically spoke about the very different intracellular traffic characterizing the different bacterial species investigated so far, that sometimes includes co-localization with endoplasmic reticulum markers (*Waddlia & Simkania*), with Golgi markers (*Chlamydia & Estrella*) or with markers of the endocytic pathway (*Parachlamydia*) [2–4]. This highlights the huge difference that exists between the different family-level lineages. G. Greub also summarized current knowledge on chlamydial division that appears to rely on RodZ & MreB [5–7]. He then spoke about persistence and aberrant bodies, as well as new insight gathered from genomics. Finally, G. Greub summarized the approaches that may be used to try to identify new T3SS effectors, which includes (i) using different bioinformatic algorithms trained with known effectors, (ii) using chaperones as baits to recover the interacting effectors, and (iii) comparing the composition of the cytoplasmic fraction of the eukaryotic cell with and without cell permeabilization with perfringolysin.

In the subsequent talk, **Mirja Puollakainen** (Finland) highlighted how the current understanding of chlamydia diversity recently increased, with a huge biodiversity of chlamydia-related bacteria. Based on serological studies, she concluded that humans are commonly exposed to *Waddlia* and *Criblamydia* but less commonly to other chlamydia-related bacteria. This may happen from direct zoonotic exposure, since animals including bats are commonly infected. It may also follow indirect exposure to ticks or interhuman transmission following respiratory tract infection. Indeed, several hints supports the role of this bacterial species as an agent of lower respiratory tract infections, at least in immunocompromised subjects.

Since ticks were recently shown to be infected by different chlamydia related-bacteria [8], **Ludovic Pilloux** (Switzerland) investigated whether tick cell lines may be used to grow these strict intracellular bacteria. He demonstrated the ability of *Estrella* and *Waddlia* to grow in tick cell lines. The subsequent talk, given by **Delaney Burnard** (Australia) was about an interesting documentation of *Rhabdochlamydiaceae* in both ticks and exposed marsupials. This report confirms the occurrence of *Rhabdochlamydiaceae* in ticks, previously documented in Switzerland and Algeria [9] and might provide a first hint on tick to mammal transmission of this family-level lineage, if MLST or genomics demonstrate that clones from ticks are similar to the strains from mammals. Finally, this session was concluded by a very nice talk from **Manon Vouga** (Switzerland) on the antibiotic susceptibility of *Simkania negevensis*, a chlamydia-related bacterium of unknown pathogenicity.

1.3. *Parallel SESSION 2 bis:* pathophysiology of Anaplasmataceae

Jere McBride (USA) first described how ehrlichiae subvert innate host defenses to escape destruction. For this, they possess tandem repeat effectors (TRPs), major immune-reactive proteins that interact with a diverse array of host cell targets, notably the Notch signaling pathway that is activated by TRP120. TRPs stimulate phagocytosis in macrophages as well as cytoskeletal reorganization/cell plasticity. The knockdown of TRPs dramatically influences ehrlichial infection and survival [10].

Nahed Ismail (USA) pointed out the imbalance between protective immunity and immunopathology associated to fatal cases of human monocytic ehrlichiosis. She notably described how an excess in cytokine and chemokine production results in a pathogenic response. Virulent *Ehrlichia* triggers canonical and non-canonical inflammasome activation. In addition, TLRs/MyD88 promote immunopathology, resulting in enhanced susceptibility to fatal ehrlichiosis and liver injury [11].

Among short talks, **Alejandro Cabezas-cruz** (France) described the accidental discovery of *Ehrlichia minasensis*, a new species described in 2016 in Brazil, in *Rhipicephalus* ticks collected on cattle. This species is phylogenetically close to *E. canis* but is pathogenic for cattle. Then, **Tian Luo** (USA) also emphasized the role of TRPs in the pathogenesis of *Ehrlichia chaffeensis*. TRPs exhibit species-specific epitopes and can interact with major cellular processes. The knock-down of TRPs affects many interacting proteins (Notch from the cell signaling pathway, vesicle trafficking and intracellular transport, actin skeleton organization, transcriptional regulation, metabolism, posttranscriptional modification, cell apoptosis), resulting in 85% of inhibition and 15% of promotion. Further, **Sudha Velayutham** (USA) detailed how human anti-Omp1 monoclonal antibodies inhibit *E. chaffeensis* infection. **Telmo Graca** (USA) explained how the hypervariable region of the outer membrane protein Msp2 from *Anaplasma marginale* accepts segments from various alleles without losing structural coherence across conserved alleles. Finally, **Anne-Claire Lagree** (France) described the role of the *Anaplasma phagocytophilum* vacuolar membrane protein in cattle abortion. By studying using VNTR strains from 123 cows including 25 that aborted, she could demonstrate that the triple-repeat in the APV-A VNTR was a reliable marker of abortion.

1.4. *SESSION 3:* Bartonella epidemiology

Henri-Jean Boulouis (France) first reported a study of 128 *Bartonella henselae* strains that could be classified into two genotypes, 1 or 2, using MLVA. They could also be clustered into two

groups, A and B, depending on their zoonotic implication. Group A strains were from cats only and were non zoonotic strains whereas genotype B strains were isolated from cats, dogs and humans and were thus zoonotic strains. Comparison of genomes from group A and B strains enabled identification of few differences including hypervariable fragments, one of which being an autotransporter acidic repeat protein. In addition, group B strains have a complete ARP gene that is expressed whereas group A strains have an incomplete ARP gene that is not expressed.

Bruno Chomel (USA) detailed the results of a survey of *B. henselae* infections in an animal shelter in San Francisco, showing a high prevalence in cats. Pr Chomel then presented data, also demonstrating a high prevalence of *Bartonella* infection in selected bat species. In France, *Pipistrella* sp. and *Myotis* sp. are especially infected by a *Bartonella* sp. close to *B. mayotimonensis*. Among vampire bats from Mexico, 38% of hematophagous, 16% of insectivorous and 4% of frugivorous bats are infected with *Bartonella* species [12].

Aranzazu Portillo (Spain) reported the detection of *Bartonella* bacteremia in asymptomatic people using an enriched blood culture system. In 97 asymptomatic patients, a seroprevalence of 82.5% was observed. A total of 20 patients were also culture-positive, three of whom were seronegative. Three species were identified including *B. henselae*, *B. vinsonii* subsp. *berkhoffii* and *Bartonella quintana*. **Richard Hassal** (United Kingdom) studied the influence of host demography on the genetic diversity of *Bartonella* in water voles. Using MLST, Dr HASSAL observed 5 genotypes of *B. grahamii*, 5 genotypes of *B. taylori* and 4 genotypes of *B. doshiae*.

1.5. *Parallel SESSION 3 bis:* genomics and genetics of Chlamydiaceae

In the first talk of the genomics session, **Adam Polkinghorne** (Australia) nicely summarized the main information gathered on chlamydia and chlamydia-related bacteria since 1998, when the first *Chlamydia* genome was sequenced [13]. The number of genomes then increased exponentially providing a unique opportunity to study in details the evolution of the Chlamydiaceae family, showing the relatively recent emergence of *C. psittaci* [14]. He also discussed the main differences between the different family-level lineages, underlying the much larger genomes of chlamydia-related bacteria. In addition to inspiring thoughts on the biology of Chlamydiales, he also questioned some specific technological aspects allowing performing the genomes of these strict intracellular bacteria directly from the clinical samples despite <1% of reads are of chlamydial origin. Immunomagnetic separation coupled to multiple displacement amplification (MDA), RNA probe hybridization and host methylated DNA depletion coupled to MDA are the tree main approaches used so far. Among others, he underlined the fact that fish pathogens exhibit very small genomes alike Chlamydiaceae, suggesting that these short genomes may correlates with a more narrow host range.

The second plenary talk of the session was given by **Ian Clarke** (United Kingdom), specifically focusing on chlamydial plasmids, which are common among Chlamydiaceae being present in all species but *C. abortus*. He initially spoke about the *Chlamydia trachomatis* plasmid that encodes among other for glycogen, underlying that the chlamydia plasmid is increasing the fitness of the bacteria, as shown by the reduced growth of plasmid-less mutants. However, plasmid-less strains could also lead to pathogenic infections in mice, showing that fitness does not automatically correlated to virulence and pathogenicity, since it is only when a given inoculum is used that significant differences start to be seen. He also underlined how fast the Swedish mutant that lacks the target of two commercial PCRs spread from 2001 to 2006 in Sweden, raising a prevalence of about 60%. He finally provided some hints on how to transform chlamydia using the *C. trachomatis* plasmid, by underlying that Pgps 2, 3 and 4 (CDS 4, 5 & 6) are not essential for transformation and rather represent virulence factors.

Then, **Kenneth Fields** (USA) summarized key steps in chlamydial transformation. It started in 2011 with the successful *C. trachomatis* transformation with a plasmid by Clarke s' team [15]. In parallel, the work by Valdivia et al. [16] was also very important to obtain quite a number of chemically-induced mutants that were then phenotypically characterized (forward genetics). Then, by insertion of "TargeTron" Johnson et al. could obtain some knockdown [17]. More recently, K. Fields laboratory succeed in targeted mutagenesis by using the FRAEM technology. FRAEM stands for fluorescence-reported allelic exchange mutagenesis [18]. This technology mainly relies on a suicide m-Cherry reporter. Thus, when allelic exchange occurs, the colour changed from red to green, with initial transformants being both green & red, thus allowing easy screen of successful exchanges.

Finally, in the last talk of the meeting, **Trestan Pillonel** (Switzerland) nicely presented his data on the comparative analysis of *Chlamydiales* genomes that enables to have new insights in chlamydial biology, using a new pan-*Chlamydiales* genomes database. As an example, he specifically discussed about the prediction of protein–protein interactions, by looking at syntenic blocks of genes, and he identified some genes that are likely implicated in the function of the T3SS, despite being still annotated as hypothetical proteins.

1.6. *SESSION 4:* pathogenesis of Chlamydia and Chlamydia-related bacteria

The first talk of this session, given by **Loyd Vaughan** (Switzerland), described the common occurrence of epitheliocystis disease in fish. This infection due to various chlamydia-related bacteria such as *Piscichlamydia* and *Clavochlamydia* is characterized by cysts occurring in fish gills [19]. However, such cysts may also occur on the fins and skin of fish, and may be due to various pathogens including a novel symbiotic gamma-proteobacteria. To further precise the pathogenesis of chlamydiae in fish, L Vaughan developed a zebrafish model of infection [20]; in that model he convincingly demonstrated that *Waddlia* may replicates, attracting mitochondria around the waddlial inclusions, as expected from living *Waddlia* bacteria. More important, in that model, neutrophils were attracted nearby to the locus of infection.

Allen W. Tsang (USA) then discussed about the possibility to block chlamydial infection by manipulating the EGFR receptor [21]. Indeed, *Chlamydia* induces ROS through EGFR. Sulforaphane and broccoli extracts are well known anti-oxydants but they have a paradoxical effect by activating the replication of chlamydiae. Conversely spilanthol, another anti-oxydant, has a very good inhibitory effect on chlamydiae, reducing the IC50 down to 41 micromol. The effect of spilanthol is likely mediated by its effect on actin reorganization.

Then, in an inspiring talk, **Raphael Valdivia** (USA) nicely presented two key important *C. trachomatis* virulence factors: Tepp and Cpos. Tepp is a translocated early phosphoprotein that mediates major changes to the host cell posphoproteome [22]. Cpos is a chlamydial promoter of cell survival as demonstrated using Cpos deficient mutants that are defective for growth in A2EN cells and for survival in mice. This phenotype was associated by the induction of a Sting-mediated robust type I and type III interferon response [23].

The session ended by two short talks. First, **Nick Wheelhouse** (United Kingdom) presented the data gathered in a cow model of infection by *Waddlia chondrophila*. No abortion occurred in the 11 inoculated cows. However, a severe chorioamnionitis was

documented in one of the cow that was sacrificed during the study, suggesting that *Waddlia* may disseminate through the bloodstream infection, before causing a focal placental infection. Indeed, placenta examination of that case showed a huge replication of *Waddlia* and a severe diffuse local inflammatory infiltrate. In the final talk of the session, **Nicolas Jacquier** (Switzerland) presented recent data on the likely role of a homolog of a *Bacillus subtilis* sporulation protein in chlamydial division.

1.7. SESSION 5: other fastidious pathogens

Session 5 was dedicated to other zoonotic pathogens. First, **Max Maurin** (France) summarized the most recent information on tularemia in France. After describing the clinical forms caused by *Francisella tularensis*, he detailed its epidemiology, pointing out that in the USA the bacterium exhibits two distinct cycles, one mountainous and the second aquatic. Two genotypes co-exist in the USA, types A and B, like in Slovakia. In contrast, In Europe, Asia, Australia and Africa, only type B can be found. In the USA, the severity of tularemia varies greatly according to the causing type, with lethality rates ranging from 0% (A2), 7% (B) to 28% (A1). Pr Maurin also exposed the reservoirs and vectors of *F. tularensis*, including rodents, cats, ticks and mosquitoes (Sweden), and highlighted the cases of transmission through contaminated water ingestion or inhalation (Kosovo), suggesting a role for amoebae, or contact with soil.

Seemay Chou (USA) gave a very nice talk on the interactions of bacterial symbionts and their tick vectors. Various factors play a role in interactions between bacteria, including quorum sensing, contact-dependent, type IV secretion system and peptidoglycan that provides structural support for the cells. Bacteria possess a *Tae*1 cell wall amidase that cuts peptidoglycan and kills cells. Dr Chou identified *Tae* homologs in tick genomes, acquired from bacteria by lateral gene transfer (LGT). These tick *Tae* encoding genes can be classified into 3 or 4 families and were acquired through 6 independent LGT events. *Tae* genes in eukaryotes are functional and encode for amidases that may be regarded as domesticated toxins that play a role in host antibacterial defense [24]. In ticks, *Tae* proteins may help controlling the bacterial population but the exact mechanism of this control is uncertain and may involve direct toxicity and/or immunodulation.

Then, **Alejandro Cabezas-Cruz** (France) described the complex microflora of arthropods, a notion overlooked by PCR. Co-infection in ticks is common and may influence the virulence of some of the pathogens. As an example, Dr Cabezas-Cruz described an antifreeze protein that is produced by ticks under the stimulation of *A. phagocytophilum* and reverts the virulence of *Staphylococcus aureus*. Using a network analysis, 14,000 interactions were identified among bacteria within *I. ricinus* ticks, thus highlighting the importance and complexity of arthropod microbiota. Network building also enabled the study of abiotic factors on the composition of the tick pathobiome, notably showing that the environment plays a role on the relative importance of bacterial populations [25].

Florian Tagini (Switzerland) delivered a short talk on the pangenome of *Mycobacterium kansasii*. He compared type I (virulent) to type II (opportunistic) and type III (avirulent) strains. Type III strains exhibit a less conserved genome than type I strains. Type VII secretion system was identified as associated to virulence, together with a pathogenicity island containing paralogs of the *espA* gene. **Lesley Bell-Sakyi** (United Kingdom) then reported her experience with the establishment of new tick cell lines. She has developed and is the curator of the tick cell biobank at the University of Liverpool that contains 49 cell lines from ixodid and 5 cell lines from argasid ticks. Dr Bell-Sakyi described the long and difficult process of establishing a new cell line, a process that may take up to 7 years.

1.8. SESSION 6: pathophysiology of Q fever

Stacey D. Gilk (USA) described the sensitivity of *Coxiella burnetii* to host cholesterol levels. She also detailed the role of cholesterol in the dynamics of the phagolysosome membranes of macrophages. *C. burnetii*-infected phagolysosomes are poor in cholesterol. Therefore, increasing the phagolysosome concentration in cholesterol inhibits *Coxiella* growth. Pr GILK also demonstrated how blocking acidification rescues *C. burnetii* death. Therefore, cholesterol-altering drugs kill *C. burnetii* in a pH-dependent manner [26].

Daniel E. Voth (USA) detailed the complex intra-cellular trafficking of *C. burnetii*. Using an *ex vivo* human lung tissue model (precision cut lung slice system), Pr Voth demonstrated that type IV secretion system activity is required for phagolysosome fusion with autophagosomes. He also described how this *ex vivo* model helped to study the role of p62 that localizes in the phagolysosome in a T4SS-dependent manner and that is stabilized by *C. burnetii*. He could conclude that p62 is not required for the intracellular growth of *C. burnetii*.

Anne F.M. Janssen (The Netherlands) investigated differentially expressed genes in patients infected by *C. burnetii* and having chronic Q fever. Her objective was to identify new biomarkers for the diagnosis of Q fever. Dr Janssen identified the CXCL9 chemokine as hyperproduced in chronic Q fever. A representative of the Defense Science and Technology Laboratory (United Kingdom) then presented an aerosol model of acute Q fever in the common marmoset, an animal exhibiting a 99.6% genetic similarity to humans. **Ruud P.H. Raijmakers** (The Netherlands) concluded session 6 by reporting the case of a 73-y-o patient who presented with an unusually severe form of chronic Q fever with aneurysm infection. The patient had antinuclear antibodies and a type 2 cryoglobulinemia. Despite treatment with doxycycline and hydroxychloroquine, the patient's status worsened and he was successively administered ciprofloxacin, mycophenolic acid and prednisone. However, he developed myopathy, which required the discontinuation of antibiotics, and died.

1.9. Parallel SESSION 6 bis: type III secretion system of Chlamydiae

Agathe Subtil (France) presented current knowledge on the type three secretion system of chlamydiae. She underlined the high conservation of the secretion apparatus in the different members of the *Chlamydiales* order that contrasts with the species specificity of the secreted effectors proteins. A Subtil also underlined that a given chaperone may be used for several effectors and that a given effector may have more than one effect. Furthermore, the presence of more than 50 different effectors underline the importance of the T3SS in controlling the interaction of *Chlamydia* with its host cell, these effectors being either (i) implicated in the bacterial entry and are then already expressed by elementary bodies, before cell infection or (ii) implicated in tight control of the intracellular traffic and/or in reducing host response to chlamydial infection. Then, **Carole Kebbi** (Switzerland) summarized current knowledge on the type III secretion system of waddlia, before describing a new effector called Wace1 and its chaperone. As many as 53 putative new Waddlia effectors have been identified and some of them are currently in the process of being characterized.

1.10. SESSION 7: genomics, transcriptomics and ecology of Anaplasmataceae

The first talk of the Anaplasma session was given by **John Steven Dumler** (USA), who specifically discussed the complex interactions between *A. phagocytophylum* and neutrophils, which have opposed

effects, activation and deactivation. Practically, this tight control and cell corruption of neutrophils is achieved thank to the *Anaplasma* protein AnkA, which contains ankyrin repeats. AnkA was shown to be specifically secreted by the type IV secretion system of *Anaplasma* before locating in the host cell nucleus were it alters gene transcription by remodeling the chromatin and by modifying the eukaryote host histone H3 [27]. JS Dumler nicely showed that AnkA is binding specific sites of the human genomes that largely overlap with regions coding for genes that are upregulated. More important, the proteins implicated in apoptosis control and cytokines were also more expressed as demonstrated by proteomic analysis. Thus, AnkA appear to be a critical T4SS effector reprogramming neutrophils that improve intracellular growth of *Anaplasma* and its microbial fitness.

Then, **Yasuko Rikihisha** (USA) presented the importance of the T4SS of Ehrlichia chaffeensis. Among others, Etf-1 is an effector that induces the formation of autophagosomes, in a Rab5-dependent manner [28].

In a very dynamic and inspiring talk, **Kelly Brayton** (USA) presented data on the hypothesis that rickettsial exclusion from ticks might occur due to the presence of an endosymbiont. Practically, they studied the microbiota of ticks with and without antibiotic treatment, to cure ticks for the presence of possible symbionts. Interestingly, the proportion of *R. bellii* increased when ticks where treated with antibiotics and the rate of *Anaplasma* acquisition by ticks decreased in the presence of increasing amount of *R. bellii*.

This session was then followed by 5 short talks. First, **Christophe Noroy** (Guadeloupe) then presented a bioinformatic algorithm allowing detecting novel T4SS effectors in *A. phagocytophilum*. These effectors generally exhibit a high GC content and are present in genomes regions exhibiting a low coding density, suggesting recent acquisition by horizontal transfer. Among 244 putative predicted effectors, several effectors exhibited ankyrin domains, likely implicated in cell component manipulation. Then, **Mustapha Dahmani** (France) presented a strategy allowing detection of *Anaplasmataceae* using a real-time PCR targeting te 23S rRNA for screening coupled to genus specific *rpoB* & *groEL* PCR to confirm positive results. This approach allowed detecting several novel species lineages. **Mathilde Gondard** (Guadeloupe) presented a large study on 542 ticks from Caribbean area demonstrated the high throughput application of their molecular tools to detect rickettsia and other tick-borne pathogens. The two last talks of the session were presented by **Young-Sun Cho** (Korea) and **Jeong-Byoung Chae** (Korea) and described the results of their investigations of various tick-borne pathogens (*Anaplasma*, *Bartonella* and *Borrelia*) in raccoon dogs and wild boars, respectively.

1.11. *SESSION 8:* pathophysiology of Chlamydia

Johannes Heggemann (Germany) first discussed of OmcB, which is a major chlamydial adhesin. Noteworthy, chlamydial entry may be reduced in presence of anti-OmcB neutralizing antibodies [29]. Then, he presented the role as adhesins of Pmp21 [30] and other related polymorphic membrane proteins (Pmps). Interestingly, these Pmps may form hetero-oligomers, allowing a total of about 10^{44} combinations. Such high variability of hetero-oligomers may have been selected upon evolution to have a variety of effective adhesins that may escape immune response.

The inflammasome activation generally induces pyroptosis and the proteolytic maturation of some pro-inflammatory cytokines such as IL1beta and IL-18. **Steven Webster** (United Kingdom) demonstrated that STING and type I IFN are critical for inflammasome activation after chlamydial infection and this occurs by activating both the canonical and the non-canonical pathway [31]. This may be important by potentially participating in the chlamydial immunopathogenesis.

Richard Hayward (United Kingdom) convincingly showed the polymerization of chlamydial cells that typically express the T3SS at one pole of the coccoid bacteria while the nucleoid is located at the opposite side of the chlamydial cell. Using modern electron microscopy tools, he could also show the precise ultrastructure of the T3SS and its change when activated upon contact of the inclusion membrane. **Richard Hayward** also showed how chlamydial entry may be studied by using both confocal microscopy and electron microscopy, especially when coupling such ultrastructure investigations to use of specific Rac and Arp 2,3 inhibitors [32].

The session also included three short talks. First, **Denys Pogoryelov** (Germany) showed some hints supporting the use of the V-A ATPase of chlamydia as a drug target. Then, **Marie de Barsy** (Switzerland) described an innovative approach that allowed to detect several new transcriptional regulators in *Waddlia*, including two that appeared to likely act on the same genes and one that likely represent a new sigma factor. Finally, **Firuza Bayramova** (Switzerland) showed her work on the division of *Waddlia* and reported three new proteins that she identified to interact with RodZ, to be recruited at bacterial septum and likely implicated in the *Waddlia* division.

1.12. *Parallel SESSION 8 bis:* Epidemiology and pathophysiology of ehrlichiae

Session 8bis, dedicated to *Anaplasmataceae*, started with a talk by **Jean-Lou Marie** (France), who made a comprehensive review of the epidemiology of infections caused by *Anaplasmataceae* in animals and humans worldwide. He notably emphasized the importance of *A. marginale* infections in cattle, causing a great economic loss in the USA. He also underlined the complex epidemiological cycles of these arthropod-borne and zoonotic microorganisms. In addition, Dr Marie described exhaustively the tick vectors transmitting *Anaplasmataceae*, showing the strong correlation between the geographic distributions of tick vectors and the ehrlichioses that they transmit. Finally, Dr Marie insisted on *Candidatus* Neoehrichia mikurensis, an emerging, but as-yet uncultured pathogen distributed in several northern European countries that opened discussion on this agent and was followed by two other dedicated talks.

Then, **Janet E. Foley** (USA) gave a talk on the ecology of *Anaplasma phogocytophilum*, starting with the vectors this species worldwide and its genetic diversity, describing the various gene targets used. In the far-eastern USA, *Ixodes scapularis* and rodents are the main vector and reservoir, respectively. Interestingly, northeast virulent strains lack the *drhm* gene. In the western USA, *Ixodes pacificus* is the main vector and its reservoirs are squirrels, chipmunks, and less commonly bears or deer. In Europe, the epidemiology is more complicated, with at least 3 tick species and many reservoirs involved. Pr Foley also made the hypothesis that *A. phagocytophilum* and *A. marginale* derive from a common ancestor associated to ungulates in the old World.

Further, **Jose A. Oteo** (Spain) focused his presentation on *Candidatus* Neoehrichia mikurensis, reminding the audience of the first detection of this species in rodents in Japan in 2004 as well as the role of *I. ricinus* as its main vector. To date, *Candidatus* Neoehrichia mikurensis has been detected in ticks in 20 European countries, in some instances in co-infection with *Borrelia* species. The reservoirs include at least 7 rodent species, mostly *Apodemus* and *Microtus* sp. mice. Other animals have also been found infected, including dogs. Pr Oteo described 16 human cases of *Candidatus* Neoehrichia mikurensis infection in Europe to date, summarizing their clinical presentation. He also reported cases of asymptomatic human

infection or cases of infection presenting with Lyme-like symptoms. Finally, Pr Oteo explained the difficult laboratory diagnosis of this infection, relying on PCR due to the absence of a serology assay.

Among short talks, **Anna Moniuszko-Malinowska** (Poland) described her experience in the diagnosis of *Candidatus* Neoehrichia mikurensis human infections in Poland among 665 tick-bitten patients. The bacterium was only detected by PCR in 3 patients hospitalized with a meningitis-like syndrome. However, Dr Moniuszko-Malinowska demonstrated that all three patients had co-infections, with either A. phagocytophilum or *Babesia microti*, and unfortunately, sequencing only confirmed *A. phagocytophilum* and *E. equi* infections. **Norio Ohashi** (Japan) presented the epidemiological characteristics of *A. phagocytophilum* in Japan. He described the results of a molecular survey in 743 ticks. The bacterium was detected in 3.4% of ticks from 6 species. Pr Ohashi dedicated the second part of his talk to the results of a sero-survey in Japanese patients, finding four seropositive patients and emphasizing the difficulty to prepare a valid antigen to obtain interpretable and reproducible data. The last presentation by **Jun-Gu Kang** (Korea) summarized nicely the diagnostic methods for the detection of tick-borne pathogens in dogs from South Korea. He first described the emergence of the virus causing severe fever with thrombocytopenia (SFTS), and insisted on the diagnostic difficulties to distinguish this infection from anaplamosis. Then, he briefly detailed the diagnostic tools used (nested-PCR for tick-borne bacteria and RT-PCR and serology for SFTS).

1.13. *SESSION 9:* New aspects of Q fever

This session started by two invited lectures on the clinical presentation of Q fever. First, **Chantal Bleekers** (The Netherlands) discussed lessons from the large Q fever outbreak that occurred in the Netherlands and the importance of FDG-PET CT in the follow-up of subjects with chronic Q fever infection [33]. Then, **Mathieu Million** (France) summarized his own clinical experience of this important infection, which may also be associated with lymphoma [34].

The remaining talks presented during that *Coxiella* session were selected from abstracts. **Sonja van Roeden** (The Netherlands) presented the observed long-term complications of chronic Q fever that included among others aortic aneurysm, valve lesions and abscesses. **Donatoa Raele** (Italy) then presented his data on the presence of *Coxiella* and *Bartonella* in red-mites and hypothesized that the presence of these bacteria in red-mites may be implicated in the pathogenesis of red-mites associated dermatitis. **Raquel Alvarez-Alonso** (Spain) then showed her data on the evolution of air & environmental *Coxiella* contamination of a sheep flock during lambing season. Finally, **Hendrik-Jan Roest** (The Netherlands) presented his data obtained in an experimental model of goat infection.

1.14. *Parallel SESSION 9 bis:* epidemiology and diagnosis of Rickettsioses

In the first talk, **Idir Bitam** (Algeria) proposed a review of the state-of-the-art methods available to trap rodents in their burrows and collect their ectoparasites in Algeria. Then, he described the various flea-borne *Rickettsia* species prevalent in North Africa, including *R. felis* and *R. typhi*, the tick-borne *R. conorii*, *R. aeschlimannii*, *R. massiliae*, *R. africae*, *R. slovaca*, *R. monacensis*, *R. helvetica*, *R. rhipicephali*, *R. sibirica* subsp. *mongolitimonae*, the mite-borne *R. akari* and louse-borne *R. prowazekii*. Pr BITAM also emphasized the difficulty to survey these diseases continuously due to the limited funding.

In the next presentation, **Shuji Ando** (Japan) summarized the current knowledge on rickettsial diseases in Japan, highlighting the major role of scrub typhus and Japanese spotted fever. He also detailed the case of patient returning from India with infection by a new species closely related to *R. honei* and *Candidatus* R. Kellyi which they provisionally named « *R. indica* ». Pr ANDO then reported the results of a genome-based SNP study aiming at genotyping *R. japonica* isolates. Only three genotypes were identified, most isolates belonging to genotype I. The hypothesis for this imbalance among *R. japonica* genotypes is that genotype I recently emerged.

In the five short presentation of session 9bis, **John A.J. Prakash** (India) described severe forms of scrub typhus in India as well as a new ELISA assay that was assessed by comparison with PCR from blood. The presented study included 54 scrub typhus cases. IgM ELISA was positive in 83% of cases as compared to 42% for PCR. IgM ELISA was positive from the 7th day of onset. In contrast, PCR from blood was negative after 8 days. Therefore, Dr Prakash proposed that PCR from blood should be reserved to early-phase specimens, in the first seven days after onset, while ELISA should be preferred after this initial period. **Adama Zan Diarra** [24] described the use of MALDI-TOF MS for the identification of ticks and tick-associated bacteria in Mali. In this country, the only known *Rickettsia* species are *R. africae* and *R. aeschlimannii*. In his study, Dr DIARRA also detected *R. sibirica* subsp. *mongolitimonae*. **Ida Chung** (USA) described drawbacks of the various additives to blood for rickettsial detection. Then, she proposed a new system to preserve *R. rickettsii* in blood specimens based on the addition of ACD-A to sampling tubes, compared to EDTA and heparin. ACD-A exhibited the longest duration of viability but heparin showed the amount of detectable DNA over time. Then, **Matthew T. Robinson** (United Kingdom) made a comprehensive analysis of the factors affecting the isolation of rickettsiae from clinical specimens, based on a series of 3227 specimens obtained from 3201 patients in Thailand. The most commonly isolated species was *Orientia tsutsugamushi*. The patient age, duration of fever, duration of illness, presence of a rash, elevated antibody titers, positive PCR, type of specimen (buffy coat), individual skills of technicians and time between sampling and inoculation influenced levels of isolation. However, a multivariate analysis only identified fever >4 days and presence of a rash as individually affecting the recovery rate. In the final presentation of session 9bis, **Cecilia Kato** (USA) proposed methods to increase PCR sensitivity for *Rickettsia* sp. to 9 copies per reaction using reverse transcriptase real-time PCR targeting the ribosomal RNA from total nucleic acids. This assay exhibited in average a 100× higher sensitivity (5–8 Cts) when compared to regular PCR.

1.15. *SESSION 10:* Rickettsia, from genomics to diagnostics

The first speaker of session 10 was **Pierre-Edouard Fournier** (France) who reviewed the progresses permitted by genome sequencing since 2000 in understanding rickettsial pathophysiology. He also described the results of genomic comparisons that led to the hypothesis that virulence in *Rickettsia* species is associated to progressive genome reduction over time, both in terms of decrease in chromosome size and plasmid size and number [35]. He finally unveiled the preliminary results of an ongoing pangenomic study of *Rickettsia* species based on the comparison of 79 genomes.

Then, **Stanislav Shpynov** (Russia) proposed a new multi-parametric approach for the systematics of rickettsiae based on their genome sequences. He named this approach genomosystematics. Using a comparison of 49 genomes of *Rickettsia* species and *O. tsutsugamushi*, Pr Shpynov could classify *Rickettsia* species into 5 groups. He did refute the creation of the transitional group by

removing *R. akari* and *R. australis* from this group. *O. tsutsugamushi* was the only member of its group. The classification system proposed by Pr Shpynov accommodates not only genomic data but also epidemiological and clinical characteristics.

In the following talk, **Marina Eremeeva** (USA) made a nice overview of the ecology, pathogenesis and genetics of *R. philipii*. She first reminded the audience the story of the discovery of this species initially isolated from Californian ticks as strain 364D. Then, she described accurately the currently known geographic distribution and prevalence of *R. philipii*, prior to reporting the clinical symptoms of human infection [36]. This *Rickettsia* species causes a febrile illness associated to an inoculation eschar (multiple in 36% of cases). The infection was severe in 29% of cases. Pr Eremeeva also highlighted the difficult serological diagnosis of *R. philipii* infections due to a unique serotype that prevents cross-reactions with other closely related species.

Among short talks, **Kazuhiro Itoh** (Japan) summarized the current knowledge on the antibiotic treatment of Japanese spotted fever. Factors of good prognosis included a reduced delay between onset of symptoms and therapy, prolonged duration of antibiotics and use of a combination of antibiotics rather than monotherapy. Then, **Rong Fang** (USA) then reported her findings on the survival of *R. australis* in macrophages being supported by inhibition of IL-1β secretion. She notably emphasized the role of Atg5, a protein involved in autophagy, showing that *R. australis* stimulates Atg5-induced autophagosomes. **Isaura Simoes** (USA) summarized the factors involved in the interaction between SFG rickettsiae and macrophages. In particular, she observed that rickettsial species vary in their tropism for macrophages, a phenomenon that she assumed might be linked to virulence. Then, she described the tyrosine kinases that play a role in cell invasion. NHE and Pak1 have a key role in cell invasion by *R. conorii* but not *R. montanensis*. **Monika Danchenko** (Slovakia) described a rare *R. felis* isolate obtained from a tick in Slovakia. This isolate was established in cell culture and precisely genetically characterized. The final presentation of session 10 was given by **Allen L. Richards** (USA) who described three new mosquito-borne rickettsial isolates from the Republic of Korea. This study was based on a collection of 2248 mosquitoes. Seven mosquito pools were PCR-positive for rickettsiae. Two of these rickettsial genotypes are closely related to *R. bellii* whereas the phylogenetic position of the third one is less clear, being either close to *R. asembonensis* or *R. felis*.

1.16. *SESSION 11:* Bartonella pathophysiology

Jane E. Koehler (USA) was the first speaker in session 11. She described the general stress response (GSR) that enables *B. quintana* to adapt to the changing environments that are the human bloodstream and the human body louse gut. The GSR, activated under 28 °C, is under the control of PhyR, an anti–anti-sigma factor and RpoE, a sigma factor that is itself under the control of NepR, an anti-sigma factor. By solving the crystal structure of the PhyR-NepR complex, Pr Koehler could demonstrate that PhyR undergoes a dramatic conformational change after phosphorylation and that this change played a critical role in the adaptative response of *B. quintana* to low temperatures.

Diana G. Scorpio (USA) delivered the second talk, about the putative risk of transfusion-associated *Bartonella* infections. She stressed out the fact that there is neither any current testing of blood products for *Bartonella* species nor any recommendation on the assays that may be used for testing. The risk is clearly underestimated as demonstrated by a Brazilian study showing a prevalence of 3.2% of stored blood specimens infected by *B. henselae* or *B. clarridgeiae*. This elevated rate may reflect the frequency of asymptomatic bacteremia in contact with cats.

Then, **Oleg Mediannikov** (France) summarized his experience on the detection of *Bartonella* infections in West Africa. He first detected five *Bartonella* species in arthropods, including the new species *B. massiliensis* and *B. senegalensis* in soft ticks and *B. raoultii*, *B. mastomydis*, a *B. elizabethae*-like species and *B. saheliensis* in rodents. Then, by testing patients with unexplained fever in Senegal, he detected *B. quintana* in blood in 1% of patients, and proposed that this bacterium may be transmitted among humans by head lice and not body lice.

Two short presentations were made in this session, including that of **Algimantas Paulauskas** (Lithuania) on the detection of *Bartonella* species in 22.4% of wild cervids and 83% of deer keds in southern Norway. Prof Paulauskas identified *B. schoenbuchensis* in red deer and their keds, and *B. bovis* in moose. Then, **David A. Jaffe** reported the seroprevalence of *B. henselae* in 32.3% Indian mongooses, thus confirming the major role of this species as natural reservoir of *B. henselae*.

1.17. *Parallel SESSION 11bis:* Orienta tsutsugamushi

Jason A. Carlyon (USA) made a nice presentation on the role of ankyrin repeat-containing effectors as modulators of host cell processes and described the numerous experiments that his team performed to gain insight into this complex phenomenon. He notably described the importance of the NF-kB pathway and how Ank 1 and Ank6 inhibit NF-kB nuclear accumulation [37].

In the next presentation, **Paul Newton** (United Kingdom) presented a clinical trial of doxycycline and azithromycin in murine typhus therapy. The trial involved 216 patients with murine typhus. Three regimens were tried: doxycycline 7 days, doxycycline 3 days and azithromycin. A high level of treatment failure was seen in patients treated with azithromycin and fever clearance was obtained in a larger delay. As a conclusion, Pr Newton recommended that azithromycin should not be used for the treatment of uncomplicated murine typhus. He also questioned the management of pregnant women and patients with severe infections.

As part of short presentations, **Daniel Paris** (Switzerland) described the burden of scrub typhus in Asia through an extensive review of the literature. He studied the data of more than 143,000 cases. Dr Paris also insisted that scrub typhus is a very neglected disease, not even included in the Global Burden of neglected diseases. In Asia, the seroprevalence for scrub typhus ranges from 9 to 28%, and an increased incidence has been reported in several countries. **Ivo Elliott** (United Kingdom) then described the ecology of scrub typhus and the evolution of diagnostic tools over time. He also insisted on the partial knowledge of mite vectors and various other aspects of the disease, urging for additional work. **Christian Keller** (Germany) reported data on a new drug active on *O. tsutsugamushi*, highlighting the estimated number of one million cases annually. The 4 antibiotic classes active are tetracyclines, azithromycin, chloramphenicol and rifampicin. A new molecule, corallopyronin A acting on RNA polymerase may be valuable for scrub typhus. An animal model in BALB/c mice confirmed the good activity of CorA on *O. tsutsugamushi* by curing all animals within 2 days. No human assays have been made to date. **Manisha Biswal** (India) reported the genetic diversity of *O. tsutsugamushi* in northern India. Scrub typhus is prevalent in 22/28 Indian states. In a series of 228 patients, Dr Biswal observed a mortality of 12.8%. Using genotyping, she observed 4 genotypes, the most important being Karp-like, notably Boryong. No Kato strains were detected. The last speaker of session 11bis was **Kyle Rodino** (USA) who described the modulation of endoplasmic reticulum (ER) by *O. tsutsugamushi*. By inducing an ER stress, he could observe a beneficial effect on *O. tsutsugamushi* growth. However, the addition of amino acids restored the growth defect. Therefore, *O. tsutsugamushi* is able to modulate ER-associated degradation.

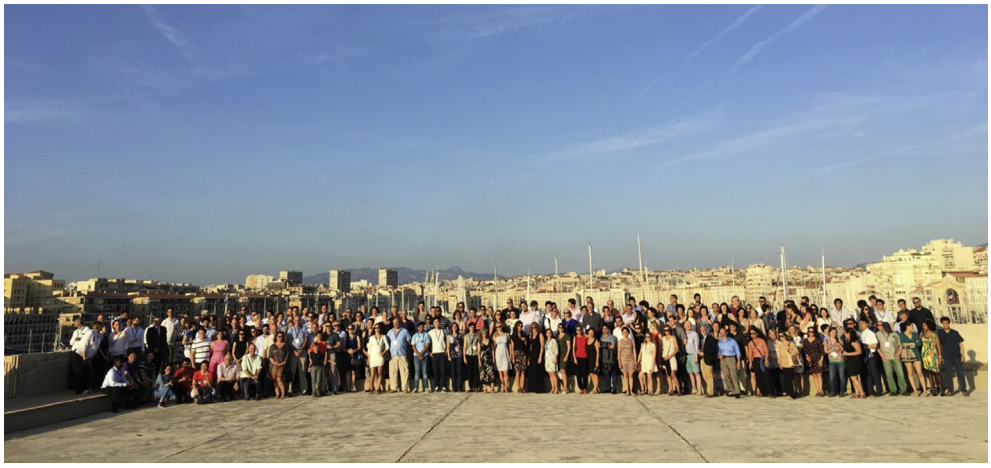

Fig. 1. All participants of the ESCCAR congress gathered at the "Vieux-Port" of Marseille for the gala dinner.

Table 1
New composition of the ESCCAR board, 2017 to 2020.

Task in the ESCCAR board	Name	Town (Country)	Speciality
President[a]	Gilbert Greub	Lausanne (Switzerland)	Chlamydia and chlamydia-related bacteria
Vice-president[b]	Aranzazu Portillo	Logrono (Spain)	Mainly Rickettsia
Past-President	Pierre-Edouard Fournier	Marseille (France)	Mainly Rickettsia & Bartonella
Secretary	Idir Bitam	Alger (Algeria)	Entomology and Rickettsia
Board member	Chantal Bleekers	Nijmegen (The Netherlands)	Coxiella & Q fever
Board member	Mirja Puoallakainen	Helsinki (Finland)	*Chlamydia pneumoniae*
Board member	Adam Polkinghorne	Brisbane (Australia)	Chlamydial genomics and veterinary microbiology
Board member	Beatte Heinrichfreise	Bonn (Germany)	Chlamydial biology

[a] The president, Mr. Prof Gilbert Greub will organize the 2020 ASR-ESCCAR meeting in Lausanne (Switzerland).
[b] The vice-president, Mrs. Dr Aranzazu Portillo will organize the 2023 meeting in Logrono (Spain).

1.18. SESSION 12: C. burnetii vaccine, diagnosis and genomics

The final session of the congress was opened by **Stephen Graves** (Australia) who described a new vaccine for Q fever. As a background, he described an outbreak of 24 Q fever cases including a patient who was vaccinated, suggesting that the degree of protection provided by the vaccine might not be optimal. Consequently, a new vaccine was developed from *C. burnetii* strain Meredith AuQ 60. When tested in Guinea pigs, vaccinated animals did not develop fever. When administered to goats, the vaccine elicited the production of antibodies but no side effects except a local transient erythema at the injection site. So this new vaccine appears very promising and will be further developed and tested.

Then, **Alice Cross** (United Kingdom) described the UK situation where no Q fever vaccine is available. Her project was to develop a *C. burnetii* LPS-based vaccine. She described the first steps of her strategy to synthesize *in vitro* the O-antigen that would be used as vaccine antigen. **Elodie Rousset** (France) described a French attempt at harmonizing the PCR-based diagnosis of Q fever in ruminants. Ten laboratories were involved in this study and seven qPCR methods tested. An assay was finally selected and its characteristics (reproducibility, sensitivity, specificity, positive and negative predictive values) assessed. Then, **Elsa Jourdain** (France) described the use of swab cloths to detect *C. burnetii* contaminating ruminant herds. Ten French departments took part in the study. Sterile swab cloths were mailed to farmers. Swab cloths were sent back to INRA where DNA extraction and PCR were performed. A total of 109 samples were obtained, 52 of which (48%) were PCR-positive for *C. burnetii*. Bacterial loads were high, up to 10P8 bacteria. These results validated this method for environmental surveillance. In the last presentation of the congress, **Dimitios Frangoulidis** (Germany) described the genomic comparison of 2 *C. burnetii* strains, virulent phase I RSA 493 and phase II RSA 439. Very few differences were identified between strains. Among those, the *wecA* gene was lost by the avirulent strain.

2. Conclusion

The ASR-ESCCAR meeting was as shown by the diversity and the quality of the talks described above a very fruitful scientific event. In addition, there were many opportunities to network during the breaks, in front of the many posters that were presented and during the social events. The latter included (i) on Monday evening, a Jazz & Blues concert given in the Toga amphitheater by a local group from Marseille mainly composed of scientists and followed by an apero served in the hall of the Faculty of Medicine at La Timone, as well as (ii) a gala dinner held in Fort Ganteaume, in a beautiful location in Marseille Vieux Port (Fig. 1). At the end of the meeting, the question that all participants were asking themselves was "when and where will be the next ASR-ESCCAR meeting" since no one would miss such a unique meeting in terms of science and networking. The answer was brought by the election of the new ESCCAR committee held on Wednesday 21st June. Prof. Greub was elected President and will thus organize with the other board members (Table 1) the next triennial international ASR-ESCCAR meeting on intracellular bacteria in Lausanne from 31th August to 3rd September 2020. Looking forward to see all of you there.

Conflict of interest

None declared.

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References

[1] Mediannikov O, Fenollar F, Bassene H, Tall A, Sokhna C, Trape JF, et al. Description of "yaaf", the vesicular fever caused by acute *Rickettsia felis* infection in Senegal. J Infect 2013;66:536–40.
[2] Croxatto A, Greub G. Early intracellular trafficking of *Waddlia chondrophila* in human macrophages. Microbiology 2010;156:340–55.
[3] Rusconi B, Kebbi-Beghdadi C, Greub G. Trafficking of *Estrella lausannensis* in human macrophages. Pathog Dis 2015;73.
[4] Vouga M, Baud D, Greub G. *Simkania negevensis* may produce long-lasting infections in human pneumocytes and endometrial cells. Pathog Dis 2017;75.
[5] Jacquier N, Frandi A, Pillonel T, Viollier PH, Greub G. Cell wall precursors are required to organize the chlamydial division septum. Nat Commun 2014;5:3578.
[6] Jacquier N, Frandi A, Viollier PH, Greub G. Disassembly of a medial trans-envelope structure by antibiotics during intracellular division. Chem Biol 2015;22:1217–27.
[7] Jacquier N, Viollier PH, Greub G. The role of peptidoglycan in chlamydial cell division: towards resolving the chlamydial anomaly. FEMS Microbiol Rev 2015;39:262–75.
[8] Pilloux L, Aeby S, Gaumann R, Burri C, Beuret C, Greub G. The high prevalence and diversity of Chlamydiales DNA within *Ixodes ricinus* ticks suggest a role for ticks as reservoirs and vectors of *Chlamydia*-related bacteria. Appl Environ Microbiol 2015;81:8177–82.
[9] Croxatto A, Rieille N, Kernif T, Bitam I, Aeby S, Peter O, et al. Presence of *Chlamydiales* DNA in ticks and fleas suggests that ticks are carriers of *Chlamydiae*. Ticks Tick Borne Dis 2014;5:359–65.
[10] Luo T, Dunphy PS, Lina TT, McBride JW. *Ehrlichia chaffeensis* exploits canonical and noncanonical host Wnt signaling pathways to stimulate phagocytosis and promote intracellular survival. Infect Immun 2015;84:686–700.
[11] Kader M, Alaoui-El-Azher M, Vorhauer J, Kode BB, Wells JZ, Stolz D, et al. MyD88-dependent inflammasome activation and autophagy inhibition contributes to *Ehrlichia*-induced liver injury and toxic shock. PLoS Pathog 2017;13, e1006644.
[12] Stuckey MJ, Chomel BB, de Fleurieu EC, Aguilar-Setien A, Boulouis HJ, Chang CC. *Bartonella*, bats and bugs: a review. Comp Immunol Microbiol Infect Dis 2017;55:20–9.
[13] Stephens RS, Kalman S, Lammel C, Fan J, Marathe R, Aravind L, et al. Genome sequence of an obligate intracellular pathogen of humans: *Chlamydia trachomatis*. Science 1998;282:754–9.
[14] Bachmann NL, Polkinghorne A, Timms P. Chlamydia genomics: providing novel insights into chlamydial biology. Trends Microbiol 2014;22:464–72.
[15] Wang Y, Kahane S, Cutcliffe LT, Skilton RJ, Lambden PR, Clarke IN. Development of a transformation system for *Chlamydia trachomatis*: restoration of glycogen biosynthesis by acquisition of a plasmid shuttle vector. PLoS Pathog 2011;7, e1002258.
[16] Kokes M, Dunn JD, Granek JA, Nguyen BD, Barker JR, Valdivia RH, et al. Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and reverse genetic analysis of Chlamydia. Cell Host Microbe 2015;17:716–25.
[17] Wang A, Al-Kuhlani M, Johnston SC, Ojcius DM, Chou J, Dean D. Transcription factor complex AP-1 mediates inflammation initiated by *Chlamydia pneumoniae* infection. Cell Microbiol 2013;15:779–94.
[18] Mueller KE, Wolf K, Fields KA. Gene deletion by fluorescence-reported allelic exchange mutagenesis in *Chlamydia trachomatis*. MBio 2016;7. e01817–15.
[19] Schmidt-Posthaus H, Polkinghorne A, Nufer L, Schifferli A, Zimmermann DR, Segner H, et al. A natural freshwater origin for two chlamydial species, Candidatus piscichlamydia salmonis and Candidatus Clavochlamydia salmonicola, causing mixed infections in wild brown trout (Salmo trutta). Environ Microbiol 2012;14:2048–57.
[20] Fehr AG, Ruetten M, Seth-Smith HM, Nufer L, Voegtlin A, Lehner A, et al. A zebrafish model for Chlamydia infection with the obligate intracellular pathogen *Waddlia chondrophila*. Front Microbiol 2016;7:1829.
[21] Patel AL, Chen X, Wood ST, Stuart ES, Arcaro KF, Molina DP, et al. Activation of epidermal growth factor receptor is required for *Chlamydia trachomatis* development. BMC Microbiol 2014;14:277.
[22] Carpenter V, Chen YS, Dolat L, Valdivia RH. The effector TepP mediates recruitment and activation of phosphoinositide 3-kinase on early *Chlamydia trachomatis* vacuoles. mSphere 2017;2.
[23] Sixt BS, Bastidas RJ, Finethy R, Baxter RM, Carpenter VK, Kroemer G, et al. The *Chlamydia trachomatis* inclusion membrane protein CpoS counteracts STING-mediated cellular surveillance and suicide programs. Cell Host Microbe 2017;21:113–21.
[24] Chou S, Daugherty MD, Peterson SB, Biboy J, Yang Y, Jutras BL, et al. Transferred interbacterial antagonism genes augment eukaryotic innate immune function. Nature 2015;518:98–101.
[25] Estrada-Pena A, de la Fuente J, Ostfeld RS, Cabezas-Cruz A. Interactions between tick and transmitted pathogens evolved to minimise competition through nested and coherent networks. Sci Rep 2015;5:10361.
[26] Mulye M, Samanta D, Winfree S, Heinzen RA, Gilk SD. Elevated cholesterol in the *Coxiella burnetii* intracellular niche is bacteriolytic. MBio 2017;8.
[27] Rennoll-Bankert KE, Dumler JS. Lessons from *Anaplasma phagocytophilum*: chromatin remodeling by bacterial effectors. Infect Disord Drug Targets 2012;12:380–7.
[28] Lin M, Liu H, Xiong Q, Niu H, Cheng Z, Yamamoto A, et al. *Ehrlichia* secretes Etf-1 to induce autophagy and capture nutrients for its growth through RAB5 and class III phosphatidylinositol 3-kinase. Autophagy 2016;12:2145–66.
[29] Moelleken K, Hegemann JH. The Chlamydia outer membrane protein OmcB is required for adhesion and exhibits biovar-specific differences in glycosaminoglycan binding. Mol Microbiol 2008;67:403–19.
[30] Luczak SE, Smits SH, Decker C, Nagel-Steger L, Schmitt L, Hegemann JH. The *Chlamydia pneumoniae* adhesin Pmp21 forms oligomers with adhesive properties. J Biol Chem 2016;291:22806–18.
[31] Webster SJ, Brode S, Ellis L, Fitzmaurice TJ, Elder MJ, Gekara NO, et al. Detection of a microbial metabolite by STING regulates inflammasome activation in response to *Chlamydia trachomatis* infection. PLoS Pathog 2017;13, e1006383.
[32] Dumoux M, Nans A, Saibil HR, Hayward RD. Making connections: snapshots of chlamydial type III secretion systems in contact with host membranes. Curr Opin Microbiol 2015;23:1–7.
[33] Barten DG, Delsing CE, Keijmel SP, Sprong T, Timmermans J, Oyen WJ, et al. Localizing chronic Q fever: a challenging query. BMC Infect Dis 2013;13:413.
[34] Melenotte C, Million M, Audoly G, Gorse A, Dutronc H, Roland G, et al. B-cell non-Hodgkin lymphoma linked to *Coxiella burnetii*. Blood 2016;127:113–21.
[35] Diop A, Raoult D, Fournier PE. Rickettsial genomics and the paradigm of genome reduction associated with increased virulence. Microb Infect 2017 Dec 27. https://doi.org/10.1016/j.micinf.2017.11.009. pii: S1286-4579(17)30216-2. [Epub ahead of print]. PMID: 29287988.
[36] Padgett KA, Bonilla D, Eremeeva ME, Glaser C, Lane RS, Porse CC, et al. The eco-epidemiology of pacific coast tick fever in California. PLoS Neglected Trop Dis 2016;10, e0005020.
[37] Evans SM, Rodino KG, Adcox HE, Carlyon JA. *Orientia tsutsugamushi* uses two Ank effectors to modulate NF-kappaB p65 nuclear transport and inhibit NF-kappaB transcriptional activation. PLoS Pathog 2018;14, e1007023.