
ННЦ "ІЕКВМ" NSC "IECVM"
НААН NAAS
UNIVERSITAS TURICENSIS
MDCCC XXXIII
EMAC·5
Odesa·October,3-5·2018
5TH EUROPEAN MEETING
ON ANIMAL CHLAMYDIOSES
AND ZOONOTIC IMPLICATIONS
FINAL PROGRAMME
AND
BOOK OF ABSTRACTS

EMAC-5 SUPPORTED BY

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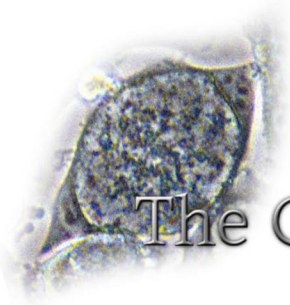

The Chlamydia Biobank

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Dear attendees of the EMAC-5,

It is a great pleasure and honor for the local Organizing Committee of this event to welcome you at the Atlantic Hotel in the attractive Ukrainian city of Odessa from 3rd to 5th October 2018. The National Scientific Center Institute of Experimental Clinical Medicine (Kharkiv, Ukraine) in association with the Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich (Switzerland) with support of the Swiss National Foundation cordially invite you to visit this beautiful place with its own special spirit on the Black Sea coast.

111 years ago, Halberstaedter and von Prowazek firstly described chlamydia-like organisms, and *Chlamydia* is still a big problem of major public health all over the world. The impact on animal and human health is tremendous and the eradication and control of this zoonotic disease remains a global and interdisciplinary challenge. Traditionally, the EMAC meeting is a good platform not only for veterinary scientists from different fields of expertise regarding Chlamydiosis, but also for junior researchers and other stakeholders who will find it a highly stimulating and inclusive environment for scientific discussions, continuing education, professional orientation and important information.

A broad spectrum of cutting-edge topics will be presented by internationally renowned keynote speakers and leading researchers. EMAC-5 sessions will cover all aspects on Pathogenesis, Diagnostics, Epidemiology of chlamydial infections as well as current knowledge in chlamydial vaccines and immunity. The program includes also poster flash presentations and a poster session.

We would like to thank the EMAC-5 Scientific Committee for their invaluable help in preparing a very interesting program. We would also say that this meeting could not be possible without support of the Swiss National Foundation and other sponsors. Most of all we thank you, the EMAC-5 participants, for your contributions and your presence. We wish you fruitful work and an inspiring meeting.

On behalf of the Organizing Committee,
Prof. Borys Stegniy
Prof. Anton Gerilovych
Dr. Vitaliy Bolotin

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VENUE

ATLANTIC GARDEN RESORT HOTEL,
24a Genuezskaya Street,
Odesa 65009 Ukraine
Phone : +38- 048-774-44-44
Fax: +38-048-774-44-45
http://atlantic-hotel.com.ua/en/

SCIENTIFIC PROGRAMME

ORAL PRESENTATIONS

Invited Lectures: 30 min + 10 min discussion
Other Speakers: 15 min + 5 min discussion

Wednesday, October 03, 2018

16:00–17:30 European Society for Animal Chlamydioses and associated Zoonoses (ESACZ) (closed meeting)

18:00–20:00 Registration

19:00–21:00 Welcome Cocktail

Thursday, October 04, 2018

08:00–08:30 Registration

08:30–08:40 Opening Address
PROF. BORYS STEGNIY — Director of NSC IECVM, Kharkiv, Ukraine

Session 1: Cell Biology I
Chairs: Ian Clarke, David Longbottom

08:40–09:20 BARBARA SUSANNE SIXT, Umeå, Sweden
Invited Lecture *Chlamydia* and host cell death — old topic, new challenges

09:20–09:40 Kensuke Shima, Luebeck, Germany
The genetic transformation of *Chlamydia pneumoniae*

09:40–10:00 Alison Favaroni, Germany
The role of polymorphic membrane proteins (Pmps) in *Chlamydia psittaci* strains with different infectious potential

10:00–10:20 COFFEE BREAK

Session 2: Cell Biology II, tissue tropism, resistance, treatment
Chairs: Daisy Vanrompay, Karine Laroucau

10:20–10:40 Robert Schoborg, Johnson City, TN, USA
Binding of elementary bodies the opportunistic fungal pathogen *Candida albicans* inhibits *Chlamydia trachomatis* infectivity

10:40–11:00 Firuza Bayramova, Lausanne, Switzerland
Characterization of cell division mechanisms of *Waddlia chondrophila*, a *Chlamydia*-related bacterium

11:00–11:20 Delia Onorini, Bologna, Italy
The influence of centrifugation and incubation temperature on various veterinary and human chlamydial species

11:20–11:40 Elisabeth Liebler-Tenorio, Jena, Germany,
Immunohistochemical demonstration of the tissue tropism of *Chlamydia pecorum* in porcine abortions

11:40–12:00 Christiane Schnee, Jena, Germany
Understanding tetracycline resistance in *Chlamydia suis*

12:00–12:20 Jasmin Kuratli, Zurich, Switzerland
wIRA/VIS irradiation reduces chlamydial infectivity of *C. trachomatis* independent of targeted cytokine and chemokine inhibition

12:20–13:20 LUNCH BREAK

Session 3: Vaccines
Chairs: Maria-Rosa Caro, Bryan Markey

13:20–14:00 PETER TIMMS, University of the Sunshine Coast, Australia
Invited Lecture Towards better control of chlamydial infection and disease in koalas

14:00–14:20 Karine Laroucau, Maisons-Alfort, France
What is the importance of abortions due to the live vaccine *C. abortus* 1B strain in sheep flocks?

14:20–14:40 Morag Livingstone, Penicuik, UK
Development of a new vaccine to protect sheep from ovine enzootic abortion

14:40–15:00 Laura del Rio, Murcia, Spain
Effect of progesterone on the vaccination and immune response against *Chlamydia abortus* in the natural host

15:00–15:20 Jizhang Zhou, Lazhou City, China
Study on an inactivated vaccine against *Chlamydia abortus* isolated from yaks (*Bos grunniens*) and immunization tests in yaks

15:20–15:45 COFFEE BREAK

15:45–16:00 The Chlamydia Biobank, University of Southampton (Colette O'Neill)

16:00–17:00 Poster Session

18:00–22.30 ODESA CITY TOUR AND SOCIAL DINNER

Friday, October 05, 2018

Session 4: Pathogenesis, animal models
Chairs: Christiane Schnee, Simone Magnino

09:00–09:40 ROBERT SCHOBORG, Johnson City, TN, USA
Invited Lecture When pathogens don't share well: Chlamydia/Herpes Simplex Virus Co-infection in cell culture and *in vivo*

09:40–10:00 Monimul Islam, University of the Sunshine Coast, Australia
Development of *Chlamydia pecorum* induced arthritis in lambs

10:00–10:20 Anne Ammerdorffer, Amsterdam, The Netherlands
Waddlia chondrophila: from bovine abortion to human miscarriage

10:20–10:40 Cheng He, Beijing, China
Feed-borne *Bacillus cereus* aggravates respiratory distress of *Chlamydia psittaci*

10:40–11:00 COFFEE BREAK

11:00–11:20 European Society for Animal Chlamydioses and associated Zoonoses (ESACZ), David Longbottom

11:20–12:00 ***Poster flash presentations: 3 min presentation, 2 min discussion***
Chairs: Yvonne Pannekoek, Nicole Borel

PF 1 The Human Immunodeficiency Virus (HIV) drugs dolutegravir and elvitegravir inhibit *Chlamydia trachomatis* and *C. muridarum* development in culture (Robert Schoborg)

PF 2 Plasmid loss in *Chlamydia muridarum* (Emma Cousins)

PF 3 Clinical outcome of ocular infection with *Chlamydia suis* S45 in nursery piglets (Christine Unterweger)

PF 4 Effect of sex hormones on the establishment of the immune response induced by vaccination against *Chlamydia abortus* in a mouse model (Maria Rosa Caro, Laura del Rio)

PF 5 Efficacy of a new inactivated vaccine against *Chlamydia abortus* and *Salmonella enterica* serovar Abortusovis experimental challenges of pregnant ewes (Carlos Montbrau Morcillo)

PF 6 Protective efficacy of a new inactivated vaccine against *Chlamydia abortus* in a pregnant mouse model (Carlos Montbrau Morcillo)

PF 7 Genomic analysis of pmps and plasticity zone of two *C. pecorum* isolates from a chamois and a water buffalo (Sara Rigamonti)

12:00–13:00 LUNCH BREAK

Session 5: Epidemiology, Genomics
Chairs: Danijela Horvatek-Tomic, Krzysztof Niemczuk

13:00–13:20 Janine Fritschi, Zurich, Switzerland
Chlamydiales and hemotropic mycoplasmas in captive and free-living bats in Switzerland, Germany and Costa Rica

13:20–13:40 Prisca Mattmann, Zurich, Switzerland
An update on *Chlamydiaceae* in wild, feral and domesticated pigeons in Switzerland

13:40–14:00 Marloes Heijne, Lelystad, The Netherlands
Rapid transmission of *Chlamydia gallinacea* in a layer hen flock

14:00–14:20 Monika Szymańska-Czerwińska, Pulawy, Poland
Whole-genome sequencing of avian *Chlamydia abortus* strains isolated from wild birds

14:20–14:40 David Longbottom, Pencuik, UK
Genomic analysis of an intermediate *C. psittaci–C. abortus* strain

14:40–15:00 Yvonne Pannkoek, Amsterdam, The Netherlands
Population structure, phylogeny and functional genomics of *Chlamydiales*

15:00–15:20 COFFEE BREAK

Chairs: Anton Gerilovych, Vitaliy Bolotin

15:20–16:00 SERVAAS A. MORRÉ, Amsterdam, The Netherlands
Invited Lecture The role of host genetic markers in the susceptibility to and severity of Chlamydiae and chlamydia-like infections

16:00–16:20 CLOSING REMARKS AND AWARDS

17:00–19:00 WINE TOUR

POSTER SESSION

Thursday, October 04, 16:00–17:00
(Posters will be displayed throughout the entire meeting)

Poster flash presentations: **1-7**
Poster Chlamydia: **8-11**
Poster other bacteria: **12-15**

	Authors	Title
1.	YAKOOB, H.; LIU, C.; GRIMM, M.; SLADE, J.; BERRY, A.; KINTNER, J., WHITTIMORE, J.; SCHOBORG, R.	The Human Immunodeficiency Virus (HIV) drugs dolutegravir and elvitegravir inhibit *Chlamydia trachomatis* and *C. muridarum* development in culture
2.	COUSINS, E.; O'NEILL, C.; MARSH, P.; CLARKE, I. N.	Plasmid loss in *Chlamydia muridarum*
3.	UNTERWEGER, C.; SETUDEH, S.; INIC-KANADA, A.; STEIN, E; BARISANI-ASENBAUER, T.; LADINIG, A.	Clinical outcome of ocular infection with *Chlamydia suis* S45 in nursery piglets
4.	MURCIA-BELMONTE, A.; ÁLVAREZ, D.; ORTEGA, N.; NAVARRO, J. A.; GÓMEZ-LUCÍA, E.; BUENDÍA, A. J.; DEL RIO, L.; SALINAS, J.; CARO M. R.	Effect of sex hormones on the establishment of the immune response induced by vaccination against *Chlamydia abortus* in a mouse model
5.	FONTSECA, M.; MONTBRAU, C.; SALINAS, J.; CARO, M. R.; GUTIERREZ-EXPOSITO, D.; ARTECHE, N.; PEREZ, V.; BENAVIDES, J.; BEZOS, J.; GARCÍA-SECO, T.; DOMÍNGUEZ L.; SITJÀ, M.; MARCH, R.	Efficacy of a new inactivated vaccine against *Chlamydia abortus* and *Salmonella enterica* serovar Abortusovis experimental challenges of pregnant ewes
6.	MONTBRAU, C.; FONTSECA, M.; ROCA, M. ORTEGA, N.; DEL RÍO, L.; ALVAREZ. D.; MURCIA-BELMONTE, A.; CARO, M. R.; SALINAS, J.; MARCH, R.; SITJÀ, M.	Protective efficacy of a new inactivated vaccine against *Chlamydia abortus* in a pregnant mouse model
7.	RIGAMONTI, S.; FLORIANO, A. M.; LONGBOTTOM, D.; SCALTRITI, E.; COMANDATORE, F.; CASADEI, G.; CAPUCCI, L.; DONATI, M.; VICARI, N.; MAGNINO, S.	Genomic analysis of pmps and plasticity zone of two *C. pecorum* isolates from a chamois and a water buffalo

	Authors	Title
8.	VAN DOOREMALEN, W.; MORRÉ, S. A.; VAN DER WIELEN, P.; AMMERDORFFER, A.	Presence of *Waddlia chondrophila* in drinking water systems in the Netherlands
9.	CASPE, S. G.; LONGBOTTOM, D.; MILNE, E.; LIVINGSTONE, M.; WATTEGEDERA, S.; AITCHISON, K.; ENTRICAN, G.; SARGISON N.; CHIANINI, F.	Why are there different clinical outcomes in multifetal sheep infected with *Chlamydia abortus*?
10.	HORVATEK TOMIC, D.; QUILICOT, A. M.; GOTTSTEIN, Z.; LUKAC, M.; PRUKNER-RADOVCIC, E.	Presence of *Chlamydiaceae* in different birds kept in zoos and other collections in Croatia
11.	KRIVOSHEI, I.; PESCH, T.; PRÄHAUSER, B., POSPISCHIL, A.; BOLOTIN, V.; BOREL, N.	*Chlamydia abortus* and *Chlamydia pecorum* infections in ruminants in Ukraine
12.	OREKHOVA, G.; ZAVGORODNIY, A.; BOLOTIN, V.	Serological screening for *Yersinia enterocolitica* serotypes O:3, O:6.30 and O:9 in ruminants and pigs during 2015–2017
13.	ZLENKO, O.; DURR, A.; SCHWARZ, J.; VYDAIKO, N. B.; GERILOVYCH, A. P.	MLVA analysis of *Franchisella tularensis* samples collected on the territory of Ukraine from 1997 to 2016
14.	MYKHAILENKO, A.; SCHWARZ, J.; DUERR, A.; BOLOTIN, V.; GERILOVYCH, A.	Characterization of Ukrainian *Brucella* strains by a Bruce-ladder multiplex polymerase chain reaction assay
15.	BILOIVAN, O.; DUERR, A.; SCHWARZ, J.; STEGNIY, B.; SOLODIANKIN, O.; GERILOVYCH, A.	Validation of qPCR method for detection of *Bacillus anthracis* pXO2 plasmid at NSC IECVM

KEYNOTE SPEAKERS

BARBARA S. SIXT is a young investigator who has recently accepted a position as research group leader at the Laboratory for Molecular Infection Medicine Sweden (MIMS), the Swedish node of the Nordic EMBL partnership, at Umeå University. While her pre-doctoral research focused on environmental *Chlamydia*-like species that naturally infect protozoa or arthropods, her most recent work builds upon the emerging genetic tools for pathogenic *Chlamydia* spp. and strives to identify and characterize *Chlamydia* virulence factors that counteract host cell death and other branches of the cell autonomous defense. Her previous research was supported by highly competitive fellowships, including a DOC-fFORTE pre-doctoral fellowship from the Austrian Academy of Sciences and a Marie Curie post-doctoral fellowship from the European Union.

PETER TIMMS is Professor of Microbiology at the University of Sunshine Coast in Queensland, Australia. He is a nationally and internationally renowned microbiologist with specific expertise in the area of *Chlamydia*. His laboratory is acknowledged as the leading Australian laboratory and one of the leading groups internationally working on all aspects of chlamydial infections.

ROBERT SCHOBORG, PhD is a Professor and Vice Chair for Education in the Department of Biomedical Sciences and the Center of Excellence for Inflammation, Infectious Disease and Immunity at James H. Quillen College of Medicine. His research over the past 12 years has been focused on the pathogenesis of sexually transmitted diseases in humans — particularly those caused by *Chlamydia trachomatis* and Herpes Simplex type 2. As a PI, he has over 60 individual and joint teaching awards/honors, a 24 year history of extramural grant funding from the United States Department of Agriculture, National Institutes of Health, and other agencies, 37 peer-reviewed manuscripts, and 68 national/international meeting abstracts.

SERVAAS A. MORRÉ is working on *Chlamydia trachomatis* infections for over 20 years, with a central focus on female infertility and women's reproductive health. He is currently the Head of the Laboratory of Immunogenetics (started in 2001, Monday–Wednesday), VU University Medical Center, Amsterdam and the Director of the Institute of Public Health Genomics (IPHG) (started in 2011, Thursday–Friday), University of Maastricht. Since February 2012, he is Professor in Host-pathogen Genomics in Public Health at IPHG. Since September 2009, he coordinates the Dutch *Chlamydia trachomatis* Reference Laboratory (Amsterdam) for the RIVM including specific CT research tasks together with Prof. Dr. Christian Hoebe, MD, PhD, Maastricht, NL (since January 2014). Finally, since 2015, he is Professor of Biotechnology and Immunogenetics at SHUATS, Allahabad, India. In addition, he is a serial entrepreneur in the field of (infectious) disease diagnostics, human biomarkers and personalized medicine to generate new applications in health care systems.

KEYNOTE LECTURES

Chlamydia and host cell death — old topic, new challenges

Sixt, Barbara Susanne

The Laboratory for Molecular Infection Medicine Sweden (MIMS), Department of Molecular Biology, Umeå University, Försörjningsvägen 2A, 90187 Umeå, Sweden

Exposure to severe physicochemical insults can cause the instant death of a cell. This cell death is typically accidental and non-regulated in nature. However, under physiological conditions cellular demise in animals and humans is in most instances a tightly regulated process that is executed and controlled by genetically encoded death programs (such as apoptosis, necroptosis, or pyroptosis) [1]. This so-called regulated cell death (RCD) has multiple different functions, all of which are essential for the survival and well-being of multicellular organisms. (1) RCD acts in developmental processes, such as in the shaping of tissues and organs. (2) RCD has a homeostatic role by balancing cell division with cell removal to maintain constant cell numbers. (3) RCD exerts an important immunological function by removing damaged and abnormal cells and by fighting infectious diseases [2].

It is well-known that the encounter with pathogenic microbes can lead to the death of exposed or infected cells. Yet, this cell death can have different reasons and may serve either the host or the pathogen [3]. (1) The pathogen may actively induce cell death, for instance to mediate its release from an infected host cell, its spread to deeper tissue layers, or the depletion of immune cells. In this situation, cell death can be beneficial for the microbe and the pathogen may have evolved specialized virulence factors or strategies to induce cell death. However, the execution of cell death may still partially rely on hijacked host-intrinsic RCD programs. (2) The infection may cause perturbations of the intracellular or extracellular microenvironment of the cell, such as for instance starvation, oxidative stress, DNA damage, or defects in cell division, which cannot be compensated by the cell, resulting in the induction of the cell's RCD programs. (3) The detection of the presence of a microbial invader by intracellular innate immune sensors may trigger cell death as part of a cell-autonomous defense response to block further replication and spread of the pathogen, to alert neighboring and immune cells, and to shape the subsequent immune response against the pathogen. Because these defensive or stress-induced modes of cell death may be potentially detrimental to the microbe, many pathogens have evolved strategies to avoid their induction [4].

The interaction of *Chlamydia* spp. with the host cell intrinsic molecular death machineries has been subject of investigation for several decades. Given the complex impact of cell death on infection and immunity, it is not surprising that these well-adapted obligate intracellular pathogens have evolved to modulate host cell death in both directions, pro-death and anti-death, depending on the circumstances. In this conference contribution, I will summarize current knowledge of *Chlamydia*'s way to modulate host cell survival and death, highlight persisting questions, and discuss

possible future directions that could lead us to a deeper understanding of both the mechanisms and the significance of this virulence trait. Some aspects that will be discussed in this context are briefly outlined below.

Host cell death is an integral part and the final stage of the *Chlamydia* infection cycle. *Chlamydia* infectivity in infected cell cultures is lost during the developmental transition from the elementary body (EB) stage to the reticulate body (RB) stage, reappears later at the time of RB to EB transition, and can eventually be found in the culture supernatant at the end of the infection cycle when infected cells disintegrate to release infectious bacteria [5,6]. It can be assumed that the mode by which this cell death occurs will influence the way how the dying cell and the released bacteria are perceived by neighboring cells and by the immune system. It may thus have important implications on bacterial spread and propagation, pathogenesis, inflammation and tissue damage, and the quality and nature of the subsequent immune response. Interestingly, our current knowledge of this process is rather scarce. This may be due to a combination of different factors, including for instance the former lack of a genetic system for *Chlamydia* spp., the complex nature of cell death, our limited ability to transfer knowledge gained from cell culture experiments to the infection situation *in vivo*, and the difficulty to study cell fate directly in experimentally infected animals.

Recent studies that used live cell imaging to monitor the fate of individual infected HeLa cells reported that *Chlamydia* spp. induce a necrotic form of cell death that is executed in an inside outside fashion, in which the membrane of the bacteria-containing vacuole (inclusion) ruptures first, followed by the rupture of other intracellular compartments and eventually the rupture of the host cell plasma membrane [7,8]. Studies with pharmacological inhibitors and *Chlamydia* mutants further suggested that the first step, the rupture of the inclusion membrane, is a bacteria-driven process, whereas the final step, the host plasma membrane lysis, may depend on a host-driven program [7–9]. In contrast to these findings, earlier work suggested that in some circumstances, observed in particular in primary cells, host cell death at the final stage of infection may occur by a mode of cell death that resembles apoptosis in some aspects, albeit it occurs in absence of activation of apoptotic caspases, and may enable immunologically silent spread of infection in absence of host plasma membrane rupture or release of pro-inflammatory cellular content [10–12]. Moreover, extrusion was described as an alternative non-destructive route of bacterial egress, in which parts of the bacteria-containing inclusion are ejected from infected cells in a manner that leaves the host cell alive [7].

Important questions that need to be addressed more thoroughly in future work include for instance: (1) Does infection-induced cell death observed (and well-studied) in cell culture reflect the natural mode of host cell death that occurs during infection *in vivo*? (2) Does the mode of infection-induced cell death differ during infection with different *Chlamydia* species and strains? (3) How important is bacterial egress by extrusion compared to bacterial egress via host cell death? (4) How does the mode of host cell death affect the viability, infectivity, and dissemination of the released bacteria *in vivo* and how does this compare to egress via extrusion? (5) How does the mode of host cell

death/egress affect the release of pro-inflammatory cytokines and danger signals and thus the nature and quality of the subsequent immune response and the extent of tissue damage and pathology; and how does cell death induced in uninfected bystander cells contribute to these responses? (6) Which *Chlamydia* factors mediate host cell death and how is their action regulated to ensure correct timing of cell death induction at the end of the developmental cycle? (7) Which host factors (and potentially death programs) participate in *Chlamydia*-induced host cell death? (8) Is it possible to modify the course of infection and/or pathology by modulation of the mode of *Chlamydia* egress?

While *Chlamydia* spp. induce host cell death at the end of their infection cycle to facilitate release of infectious bacteria, premature host cell death can be detrimental to the pathogen. Replication of *Chlamydia* spp. is absolutely restricted to the intracellular growth niche provided by the host cell. Moreover, the replicative intracellular developmental stage, the RB, is non-infectious and fragile [13]. Consistently, experimental induction of apoptosis at early or mid-stages of the infection cycle, when host cells contain mostly non-infectious RBs, can effectively disrupt chlamydial development [14]. Interestingly, animal-derived cells induce rapid cell death in response to infection with *Chlamydia*-related species that naturally replicate in unicellular eukaryotic host cells [15,16]. This finding indicates that host cell death can be activated as host defense response and suggests that animal/human-adapted *Chlamydia* spp. likely evolved strategies to prevent induction of this response. Indeed, recent studies exploiting the novel genetic tools for *Chlamydia* spp. demonstrated that some *Chlamydia* spp., such as *C. trachomatis,* actively counteract premature host cell death induction [17,18]. More precisely, it was shown that *Chlamydia* mutants that are deficient for certain inclusion membrane (Inc) proteins, which are secreted effector proteins that are inserted into the membrane of the *Chlamydia* inclusion, induce spontaneous premature (apoptotic and necrotic) host cell death that disrupts formation of infectious EBs. This host cell death is at least partially dependent on the intracellular innate immune sensor STING [17,18], giving further support to the idea that host cell death can be induced as part of a host cell intrinsic defense response. Moreover, while *Chlamydia* spp. primarily replicate in epithelial cells, macrophages can be infected and can respond by activation of inflammatory caspases and pyroptotic cell death; which constitutes another example of a cell-autonomous defense response that triggers the death of the infected cell [19,20].

Important questions that need to be addressed in this context in future work include for instance the following: (1) How does the presence of specific Inc proteins in the inclusion membrane prevent activation of innate immune signaling pathways and induction of cell death during infection with *C. trachomatis*? (2) How can related *Chlamydia* spp. that lack homologs of certain members of these specific Inc proteins sustain host cell viability? (3) What is the molecular basis of host cell death induced during infection with the mutants and does it resemble natural host cell death induced by *Chlamydia* spp. at the end of the infection cycle? (4) Can we interfere with the anti-death activity of these virulence factors or otherwise exploit the program of defensive

host cell death to fight *Chlamydia* infection? (5) How does pyroptosis in immune cells contribute to the control of *Chlamydia* infection and/or pathogenesis? (6) Does *Chlamydia* actively suppress innate immune signaling pathways that lead to pyroptosis?

Apart from the ability to suppress innate immune signaling that can induce cell death as cell-autonomous defense response, *Chlamydia* spp. are also known to directly interfere with the apoptotic machinery in infected cells [21]. Indeed, cells infected with *Chlamydia* spp. are protected from the induction of apoptosis upon exposure to various strong inducers that cause intracellular damage or directly induce cell death signaling pathways, such as UV irradiation, cytotoxic chemicals, and immune mediators [22,23]. Interestingly, numerous anti-apoptotic activities have been attributed to *Chlamydia* spp., including for instance the induction and stabilization of the anti-apoptotic protein Mcl-1 [24], down-regulation and degradation of p53 [25,26], and enhanced mitochondrial binding of hexokinase-II [27].

While *Chlamydia*'s anti-apoptotic trait has been known for a long time, there are still many open questions concerning the underlying molecular mechanisms and in particular its significance for *Chlamydia* replication and pathogenesis. These include for instance: (1) Which *Chlamydia* virulence factors mediate inhibition of apoptosis? (2) What is the relative importance of the different described modes of apoptosis inhibition? (3) What is the nature and strength of pro-apoptotic stimuli that infected cells would typically encounter *in vivo*? (4) Given the high complexity of cell death and crosstalk between death programs, are infected cells indeed protected from pro-apoptotic stimuli in a way that enables them to sustain normal replication and development of intracellular *Chlamydia* under pro-apoptotic conditions? (5) Does *Chlamydia* infection also directly modulate other death programs, such as for instance the molecular machineries that execute necroptosis or pyroptosis, and if yes, how?

Altogether, it can be summarized that, although *Chlamydia*'s way to modulate host cell death has been subject of extensive investigations for many years, our understanding of the underlying molecular principles and in particular of its significance for disease and pathology is still insufficient. While it can be expected that the new tools for molecular genetic analysis of *Chlamydia* spp. will greatly enhance our mechanistic understanding at the molecular and cellular level, a major challenge for the future will be the development of experimental systems that will enable us to study the effect of host cell death modulation on pathology and immunity under physiological infection conditions.

References

1 Galluzzi, L. et al. *Cell Death Differ* 25, 486–541 (2018).
2 Jorgensen, I., Rayamajhi, M. & Miao, E. A. *Nat Rev Immunol* 17, 151–164 (2017).
3 Labbé, K. & Saleh, M. *Cell Death Differ* 15, 1339–1349 (2008).
4 Lamkanfi, M. & Dixit, V. M. *Cell Host Microbe* 8, 44–54 (2010).
5 Higashi, N., Tamura, A. & Iwanaga, M. *Ann N Y Acad Sci* 98, 100–121 (1962).
6 Friis, R. R. *J Bacteriol* 110, 706–721 (1972).

7 Hybiske, K. & Stephens, R. S. *Proc Natl Acad Sci U S A* 104, 11430–11435 (2007).
8 Kerr, M. C. et al. *Nat Commun* 8, 14729 (2017).
9 Yang, C. et al. *mBio* 6, e01648–01615 (2015).
10 Ojcius, D. M. et al. *J Immunol* 161, 4220–4226 (1998).
11 Perfettini, J. L. et al. *J Biol Chem* 278, 9496–9502 (2003).
12 Jungas, T. et al. *Microbes Infect* 6, 1145–1155 (2004).
13 Tamura, A., Matsumoto, A. & Higashi, N. *J Bacteriol* 93, 2003–2008 (1967).
14 Ying, S. et al. *J Infect Dis* 198, 1536–1544 (2008).
15 Sixt, B. S. et al. *PloS one* 7, e29565 (2012).
16 Ito, A. et al. *PloS one* 7, e30270 (2012).
17 Sixt, B. S. et al. *Cell Host Microbe* 21, 113–121 (2017).
18 Weber, M. M. et al. *Cell reports* 19, 1406–1417 (2017).
19 Finethy, R. et al. *Infect Immun* 83, 4740–4749 (2015).
20 Webster, S. J. et al. *PLoS Pathog* 13, e1006383 (2017).
21 Sharma, M. & Rudel, T. *FEMS Immunol Med Microbiol* 55, 154–161 (2009).
22 Fan, T. et al. *J Exp Med* 187, 487–496 (1998).
23 Fischer, S. F. et al. *J Exp Med* 200, 905–916 (2004).
24 Rajalingam, K. et al. *PLoS One* 3, e3102 (2008).
25 Gonzalez, E. et al. *Nat Commun* 5, 5201 (2014).
26 Siegl, C. et al. *Cell reports* 9, 918–929 (2014).
27 Al-Zeer, M. A. et al. *EBioMedicine* 23, 100–110 (2017).

Towards better control of chlamydial infection and disease in koalas

Timms, P.

University of Sunshine Coast, Sippy Downs, Queensland, Australia

The last remaining member of the *Phascolarctidae* family, the koala (*Phascolarctos cinereus*), is undergoing significant population declines throughout its range. Despite other serious threats such as habitat destruction, road accidents and dog attacks, in many populations, the main risk is from disease caused by *Chlamydia pecorum*. Declines attributed to chlamydial disease are predicted to cause localised extinction events within 5–10 years. The clinical diseases caused by *C. pecorum* infection in the koala include kerato-conjunctivitis, urinary tract disease and reproductive tract disease. The prevalence of *C. pecorum* in wild koala populations is generally high (50%) and is present across their full geographical range. Significant diversity (10–30%) of the *C. pecorum* Major Outer Membrane Protein (MOMP) has been found amongst wild koala populations. The nucleotide sequence of *C. pecorum ompA,* which has four variable domains, has been used to genotype *C. pecorum* samples collected from koalas, leading to the description of 11 koala-associated genotypes, named A–K. Some *C. pecorum* infections remain asymptomatic, while others produce a varying array of overt signs of disease. Progression to disease does not appear to be directly associated with chlamydial *ompA* genotype, but more so with the host condition. For example,

koalas co-infected with an immunosuppressive koala retrovirus exogenous variant (KoRV-B) were more likely to progress to chlamydial disease when infected than koalas that were KoRV-B negative animals.

There are a range of strategies that might be used to better control chlamydial infection and disease in wild koala populations, including, (a) better understanding of the mechanism of transmission, to enable strategies to break the transmission cycle, (b) develop new, *Chlamydia*-specific antibiotics, (c) use veterinary intervention management of whole populations, (d) understand other factors that might be contributing to infection, such as the koala's microbiome or viral infections such as koala retrovirus (KoRV), and finally, (e) the development of vaccines.

Understanding the modes of transmission of *C. pecorum* between koalas: It has previously been accepted that the main mode of transmission of *C. pecorum* infection between koalas is via sexual contact. Our recent study showed that 27% of young koalas (9–13 months of age; presumably prior to sexual contact) were already infected with *C. pecorum*. Ocular and urogenital infection levels were similar in this age group. The ratio of ocular to UGT infections decreased markedly in older koalas, suggesting that after early mother-to-young transmission, sexual contact becomes the main mode of transmission. This information potentially affects the strategies used for control.

Development of new anti-*Chlamydia* antibiotics: While currently used antibiotics such as chloramphenicol, are effective against koala *Chlamydia* strains *in vitro*, there are several negative aspects for their use *in vivo*. The koala has effective enzymes to degrade xenobiotics, such as those present in eucalypt leaves, but also against antibiotics. In addition, the extended administration of broad spectrum antibiotics causes significant dysbiosis of the koala's gut microbiome, which alone is a significant health issue. Recent work is examining the development of novel anti-chlamydial drugs, such as the HtrA target, JO146. This new drug has shown promise in killing koala *C. pecorum* strains in vitro as well as in vivo.

Understand other "infections" such as koala retrovirus and host microbiome on chlamydial infection and disease: While we once may have thought that chlamydial infection alone was responsible for all chlamydial disease, it is now becoming obvious that other host factors are also major contributors. The virulence of chlamydial infection in wild koalas is highly variable between individuals. Some koalas can be infected (PCR positive) with *Chlamydia* for long periods but remain asymptomatic, whereas others develop clinical disease. *Chlamydia* in the koala has traditionally been studied without regard to coinfection with other pathogens, although koalas are usually subject to infection with koala retrovirus (KoRV). Retroviruses can be immunosuppressive, and there is evidence of an immunosuppressive effect of KoRV *in vitro*. Originally thought to be a single endogenous strain, a new, potentially more virulent exogenous variant (KoRV-B) was recently reported. We hypothesized that KoRV-B might significantly alter chlamydial disease outcomes in koalas, presumably via immunosuppression. By studying sub-groups of *Chlamydia* and KoRV infected koalas in the wild, we found that neither total KoRV load (either viraemia or proviral

copies per genome), nor chlamydial infection level or strain type, was significantly associated with chlamydial disease risk. However, PCR positivity with KoRV-B was significantly associated with chlamydial disease in koalas (p = 0.02961). This represents an example of a recently evolved virus variant that may be predisposing its host (the koala) to overt clinical disease when co-infected with an otherwise, asymptomatic bacterial pathogen (*Chlamydia*).

Development of chlamydial vaccines: We have now conducted a significant number of trials towards the development of a chlamydial vaccine for koalas (2010–2017).

The first koala anti-*Chlamydia* vaccine trial was conducted in 2010 by Carey *et al.* on 18 captive, healthy female koalas. This first vaccine consisted of a combination of three recombinant chlamydial antigens fused to a carrier protein. These antigens had previously shown an ability to provide partial protection against different chlamydial species in the mouse model. Three separate adjuvants, Immunostimulating complex (ISC), Alhydrogel and TiterMax Gold were trialed. The vaccine was administered sub-cutaneously via a three-dose regime. In the koala, TiterMax Gold was not a suitable adjuvant, due to adverse reactions at the site of injection. By comparison, ISC was safe and resulted in the highest lymphocyte proliferative response, which was sustained for the duration of the trial (270 days) as well as showing the highest genital IgG antibody response. This initial trial clearly established that koalas could mount a strong and sustained immune response to foreign antigens. The risk of inducing an inflammatory response by vaccinating animals that had previously been infected with *Chlamydia* was unknown, and previous mouse studies suggested that this might be an issue if the wrong chlamydial antigens were used. Kollipara *et al.* (2012) vaccinated 10 koalas that were not only infected with *Chlamydia* but were showing clinical signs of disease at the time of vaccination. This study found that none of the animals demonstrated any adverse reactions or worsening of the clinical signs following vaccination. This was an important trial to demonstrate the safety of using a chlamydial vaccine in both healthy and diseased koalas.

One of the major issues for the development of vaccines is the choice of antigen. Chlamydial studies in the mouse model have previously shown that the chlamydial MOMP was a major component of the chlamydial surface, highly immunogenic and could provide a level of immunity against live challenge. One disadvantage of chlamydial MOMP however is that it varies between species and strains of *Chlamydia*. Despite the diversity seen between strains of koala *C. pecorum*, rMOMP was chosen as the vaccinating antigen and evaluated in a series of trials. Initially, the single "G" variant of rMOMP was used, but subsequently a multiple subunit vaccine containing a combination of rMOMPs (the most prevalent variants found in circulation in wild koalas) has been adopted. Kollipara *et al.* (2013) assessed the cross-protective ability of three *C. pecorum* rMOMP genotypes (A, F, and G), combined with ISC adjuvant, in a vaccine trial of healthy, captive female koalas. In this study, koalas were vaccinated with either a single rMOMP genotype (A, F or G) or, for the first time a combined rMOMP A plus F genotype vaccine. It was demonstrated that circulating plasma antibodies were capable of not only neutralizing the homologous *C. pecorum* infection

in vitro but also heterogeneous *C. pecorum* infections, and a significant cross-strain lymphocyte proliferation ability was demonstrated. It was thus determined from this study that a multi-subunit vaccine containing multiple MOMPs should be the favoured formulation for future studies. A formulation of three rMOMPs (3rMOMP) A, F, and G, was selected for downstream evaluations.

Waugh *et al.* (2016) conducted the first field trial that vaccinated wild koalas in their natural habitat. The aim of this study was to determine the therapeutic value of vaccination in already infected animals, as well as the protective value of the vaccine, following natural exposure of koalas to *Chlamydia* in the wild (natural challenge study). Sixty wild koalas were separated into two groups of 30, each containing a mix of males and females, as well as a mixture of animals that were either *Chlamydia* PCR positive or negative at the time of vaccination. One group received no vaccine (control) while the other received a vaccine containing 3rMOMP (A, F, and G) combined with ISC adjuvant. The results of this trial were very promising. After a 12-month period, vaccinated koalas that were *Chlamydia* positive at the time of vaccination, were significantly more likely to reduce or maintain their chlamydial load (i.e. no increase in chlamydial infection load). By comparison, *Chlamydia* positive control koalas (not vaccinated) were significantly more likely to increase their chlamydial load in the subsequent 6 to 12 month period. Importantly, it was noted that although some chlamydia negative vaccinated koalas contracted a new infection post vaccination, they did not go on to develop disease. This contrasts with animals in the control unvaccinated group, where a significant proportion contracted new infections that progressed to severe disease pathology, and in some cases the disease state was serious enough to warrant humane euthanasia.

The results of these initial *C. pecorum* vaccine trials had shown that a vaccine consisting of 3rMOMP proteins combined with ISC adjuvant, given sub-cutaneously as a three-dose vaccination schedule, was: 1) safe to administer to both healthy and clinically diseased koalas; 2) led to the development of specific humoral and cell-mediated immune responses, and; 3) elicited a therapeutic effect on animals already infected. Whilst this formulation of the vaccine was showing promising results, the logistics of the three-dose vaccination regime remained challenging for administering in wild animals. Khan *et al.* (2014) addressed this by trialing the 3rMOMP (A, F, and G) with a novel adjuvant which only required one dose. This was a tri-adjuvant (TriAdj) comprised of polyphosphazine (PCEP), polyinosinic polycytidylic acid (poly I:C) and a host defense peptide (HDP) HH2, that has previously been used successfully in laboratory animal models. A significant immune response was maintained for 54 weeks, post vaccination. MOMP-specific IgG antibody levels, with neutralization potential, were present in plasma, ocular and urogenital secretions, and a significant lymphocyte proliferation ability was noted. This suggested that the 3rMOMP/Tri-Adj formulation resulted in a strong and long-lasting, cellular and antibody response.

Overall, the development of an anti-*Chlamydia* vaccine is showing great promise and recently, it has been shown that it can also have a positive clinical benefit in animals

already with ocular disease. Together, these strategies enable novel combined approaches to reducing the burden of chlamydial infection and disease in this unique animal.

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When pathogens don't share well: Chlamydia/Herpes Simplex Virus co-infection in cell culture and *in vivo*

Schoborg, R.[1]; Slade, J.[2]; Hall, J.[1]; Sun, J.[3]; Deka, S.[4]; Kintner, J.[1]; Phillips-Campbell, R.[5]; Duffourc, M.[1]; Dessus-Babus, S.[6]; Raulston, J. E.[7]; Wyrick, P. B.[7]

[1] Department of Biomedical Sciences, Center for Infectious Disease, Inflammation and Immunity, Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA
[2] Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA
[3] Department of Biomedical Sciences, Elson S. Floyd College of Medicine, Washington State University, Spokane, WA, USA
[4] Roche Molecular Systems, Pleasanton, CA, USA
[5] Department of Biology, Emory and Henry College, Emory, VA, USA
[6] Novartis Institutes for Biomedical Research (NIBR), Cambridge, MA, USA
[7] Department of Microbiology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA

Introduction to the Problem. Worldwide, humans acquire >1 million new sexually transmitted infections (STIs) daily. In 2008, diagnosis and treatment of STIs in the United States (US) alone cost >16 billion dollars. Herpes Simplex Virus type 2 (HSV-2) and *Chlamydia trachomatis* cause STIs, with 131 million new *C. trachomatis* infections every year and 0.5 billion total HSV-2 infections worldwide. *C. trachomatis* genital infection is the most common reportable disease in the US, with ~1.6 million new cases in 2016. There are a total of ~24 million HSV-2 infected individuals in the US, with >750,000 new infections reported annually. Because HSV-2 establishes latent infection characterized by frequent reactivation and shedding, untreated HSV-2 infected individuals may transmit the virus to naïve sexual contacts throughout their lives. Individuals with one STI are at higher risk for acquiring others and, unsurprisingly, *C. trachomatis* and HSV-2 cervicovaginal coinfections are common.

Until recently, the high incidence of multiple STIs was assumed to be primarily a behavioral issue, in that the same behaviors increase risk for different STIs. However, recent studies indicate that STI agents may interact with other pathogens, the genital microbiome, and/or the host in ways that alter acquisition frequency of other STI pathogens or modify disease outcome. As a result, over the last decade, we have carried out studies to explore whether *C. trachomatis* and HSV-2 interact, either in cell culture or *in vivo*.

Cell Culture Studies. Initially, polarized HeLa (a human cervical epithelial cell line) and HEC-1B (a human endometrial epithelial cell line) monolayers were infected with *C. trachomatis* serovar E followed by HSV-2 twenty four hours later. HSV-2 superinfection induced the chlamydiae to exit the normal developmental cycle and enter a state variably denoted in the literature as persistence, the chlamydial stress response, or the aberrant body (AB) phenotype. In this state, developing reticulate bodies (RB) exposed to a stressor cease dividing and enlarge to form AB. RB to elementary body (EB) transition is also blocked, reducing production of infectious chlamydial progeny. Finally, if the stressor is removed, AB resume dividing-producing RB and, ultimately, infectious EB. Persistence/stress is, thus, reversible and allows developing chlamydiae to remain viable inside host cells during unfavorable environmental conditions. In follow up coinfection studies, addition of cycloheximide (which inhibits HSV-2, but not chlamydial, replication) does not prevent HSV-2 induced arrest of chlamydial development. This demonstrates that productive virus replication is not required to induce *C. trachomatis* persistence/stress. Furthermore, coinfection with UV-inactivated HSV-2 initially suppressed EB production, but chlamydial infectivity recovered within 44 hours. We concluded from these data that: i) as expected, HSV-2 induced persistence/stress is reversible; and ii) HSV-2 superinfection altered chlamydial development by binding to and/or entering the chlamydia-infected host cell.

HSV-2 attaches to and enters the host cell via a complex series of sequential interactions between the viral surface proteins gB, gD, and gH/L and multiple host cell surface receptors and co-receptors. HSV-2 binding to many of these host receptors also activates cellular signaling pathways. Pre-incubation of viral particles with gD-specific neutralizing antibody prevents HSV-2 induced chlamydial persistence/stress, suggesting that HSV gD interaction with host cell surface receptors provides the stimulus necessary to alter *C. trachomatis* development. Moreover, exposure of *C. trachomatis* infected cells to soluble viral gD:Fc fusion protein decreases EB production to a degree similar to that observed in HSV-2 coinfected cultures. Therefore, we concluded that HSV gD interaction with host cell surface is sufficient to trigger a host response that restricts chlamydial development.

Nectin-1 (nec-1) is a host cellular adhesion protein that helps form adherents and tight junctions. Nec-1 also binds HSV-2 gD and is one host co-receptor required for viral epithelial cell entry. We performed coinfection experiments using nec-1 specific HSV mutants, which demonstrated that nec-1 tropic HSV variants induce chlamydial persistence. We also used siRNA to knock down nec-1 expression in stable HeLa cell

lines, which were then *C. trachomatis* infected. Importantly, ~80% nec-1 knock down induced the AB phenotype and reduced EB production in the absence of HSV coinfection. As HSV downregulates host cell nec-1 surface expression after viral entry, these data suggest that HSV-2 coinfection alters chlamydia development by reducing host nec-1 function. These studies also indicate that host nec-1 is required for normal chlamydial development in cultured genital epithelial cells.

***In vivo* Studies.** We previously observed that HSV-2 coinfection alters *C. muridarum* and *C. trachomatis* development similarly in culture. The role of nec-1 during *in vivo* infection was determined by examining both cervicovaginal and rectal *C. muridarum* infection in homozygous nec-1 knock out (KO) mice. In female mice, nec-1 KO reduced vaginal *C. muridarum* shedding by ~90% compared to wildtype control mice. These data are similar to our cell culture data and indicate that nec-1 is required for efficient *C. muridarum* genital infection in female mice. In contrast, rectal shedding from infected male mice was unaltered by the nec-1 KO, indicating that nec-1 is dispensable during rectal infection in male mice and suggesting a tissue-specific effect.

We also recently established a vaginal *C. muridarum*/HSV-2 coinfection model in female mice. In this model, female BALB/c or C57/B6 mice were cervicovaginally infected with *C. muridarum* and 3 days later with HSV-2. While 40–100% of mice singly infected with HSV-2 develop fatal neuroinvasive HSV disease, *C. muridarum*/HSV-2 co-infected mice show both reduced viral shedding and lower mortality (Figure, compare black and green lines). We conclude that chlamydial pre-infection protects mice from a subsequent lethal HSV-2 challenge. Both our, and other, studies indicate that *C. muridarum* infection induces genital tract IFN-β secretion. IFN-β binds to type I interferon receptors (IFNR), eliciting host cellular antiviral responses that inhibit HSV replication *in vitro* and *in vivo*. Therefore, we hypothesized that chlamydial pre-infection protects mice from HSV-2 challenge via the IFN-β/INFR-induced antiviral response. To test this hypothesis, we performed vaginal co-infections in IFNR knockout (IFNR KO) mice,

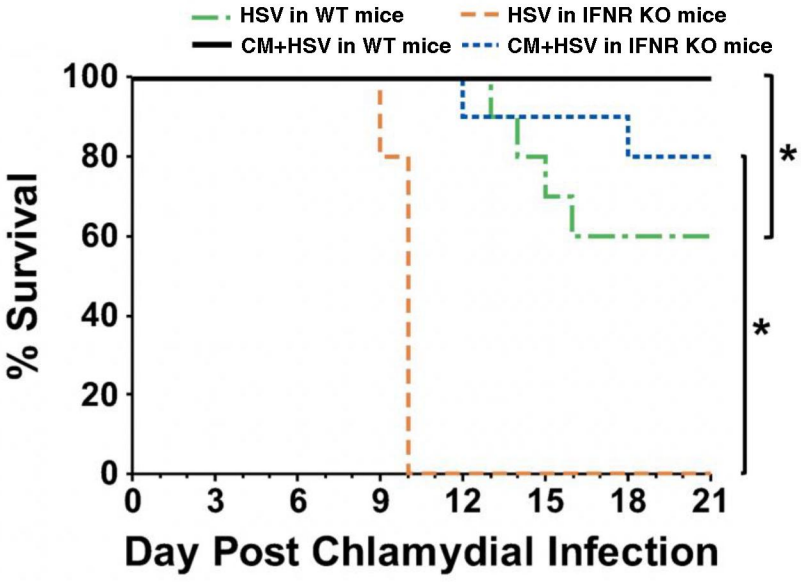


Figure. Female C57/B6 wildtype (WT) and Interferon receptor knock out (IFNR KO) mice were vaginally infected with *C. muridarum* (CM), followed by HSV-2 (HSV) 3 days later. Mice were monitored daily for signs of neuroinvasive disease and were euthanized when such signs became apparent. Percent survival was plotted vesus day post-chlamydial infection. Groups that were significantly different are marked with *.

which are deficient in IFN-β-induced antiviral responses. Surprisingly, *C. muridarum* pre-infection continued to protect from HSV-induced mortality (Figure, compare blue and oranges lines) in the absence of IFNR-mediated responses. These data indicate that IFN-β-induced antiviral responses are likely not required for the observed protective effect.

Conclusions. Over a decade of research in our laboratory has demonstrated that: i) HSV coinfection induces chlamydial persistence/stress in culture; ii) chlamydia pre-infection inhibits progression of HSV-2 neuroinvasive disease in mice; and iii) the host nec-1 protein is required for efficient chlamydia development in culture and *in vivo*. In a more general sense, this body of work illustrates how studying pathogen coinfections, using linked cell culture and *in vivo* approaches, can expose aspects of chlamydia/host cell interaction that are unlikely to have been discovered by a different approach. Future studies in our laboratory will focus on unraveling: i) the basis for the tissue-specific nec-1 requirement in rectal versus genital infection; and ii) a possible role for "trained immune" and/or cholinergic anti-inflammatory responses in chlamydia-induced protection from HSV-2 neuroinvasive disease.

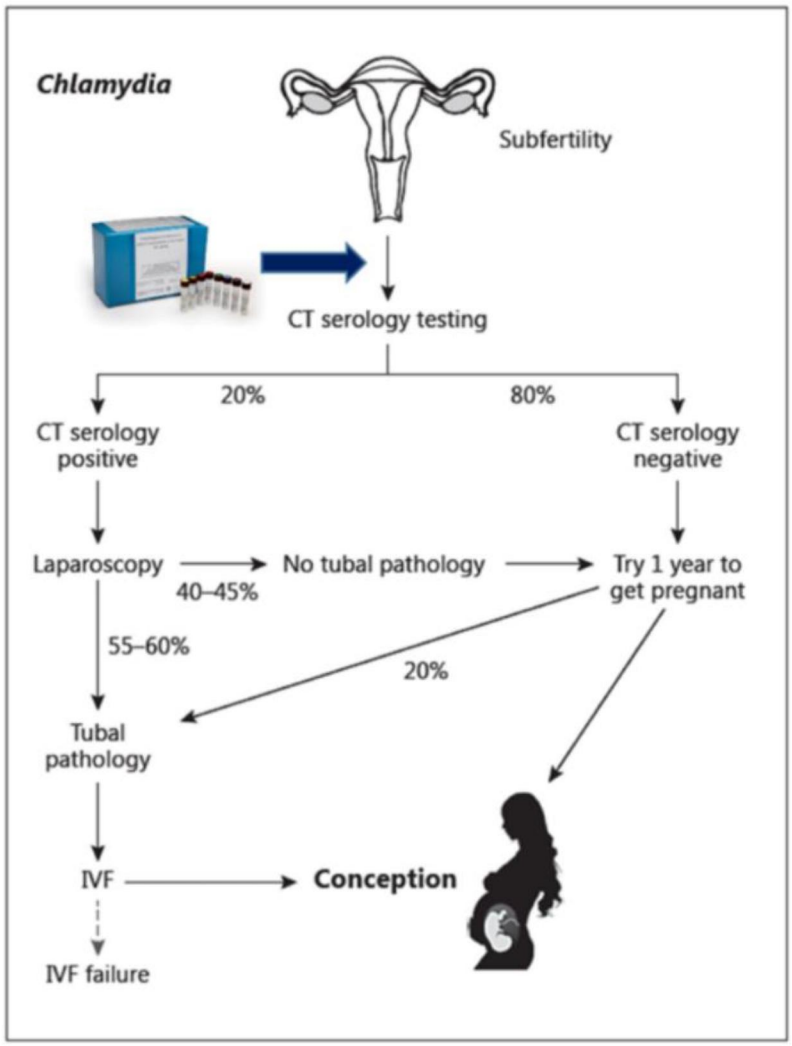
The role of host genetic markers in the susceptibility to and severity of chlamydiae and chlamydia-like infections

Prof. Servaas A. Morré, PhD[1,2,3,4]

[1] Head Laboratory of Immunogenetics, Department of Medical Microbiology and Infection Control, VU University Medical Center, Amsterdam, The Netherlands
[2] Director of the Institute for Public Health Genomics, Department of Genetics and Cell Biology, Maastricht University Medical Center, Maastricht, The Netherlands
[3] Director of the Dutch *Chlamydia trachomatis* Reference Laboratory for the Dutch National Institute of Public Health and the Environment (RIVM) and RIVM European CDC (ECDC) Dutch representative for *Chlamydia trachomatis* infections
[4] Co-Founder & Co-Director Microbiome Ltd and Founder & Director TubaScan Ltd, spin-off companies of the VU University medical center Amsterdam, The Netherlands

Research in genetic identical twins showed 40% of one's response to *Chlamydial trachomatis* (CT) is based on the host genetic make-up. Also in koalas the course of infection is in part based on the host genetic variation. Single Nucleotide Polymorphisms (SNPs) genotyping represents a powerful tool to quickly obtain information on host genetics providing insight in the immunological pathways involved, information which could lead identify for instance women at risk for late complications of CT infections. Female Reproductive Health (FRH) is affected by *Chlamydia trachomatis* a sexually transmitted infection and as shown recently influenced by the environmental *Chlamydia*-likes as *Waddlia chondrophila* which live in amoebae. It is also know from animal experiments that the female reproductive health is affected by the presence of multiple exposures of CT and Chlamydiae in

general, though it is not well studied yet. It is also known that chlamydiae in poultry (oa *C. psittaci*) is very prevalent and can be transmitted to humans. From a one health perspective it would be of interest to focus on Chlamydiae in the environment and in animals in relation to FRH with the hypothesis that having different chlamydiae present while also having a CT infection will greatly enhance the risk for infertility in women. Taking into account the host genetic markers in such an analysis will potentially also explain further why certain women (or animals) have a different course of infection while potential confounders have been taken into account.



Most advances have been made on host genetic markers in women and FRH. Almost 25% of women with *Chlamydia trachomatis* (CT) associated subfertility are faced with suboptimal treatment due to a lack of accurate diagnostic tools (see the Figure). Genetic studies have enabled the discovery of 25 single nucleotide polymorphisms (SNP) and their validation as subfertility biomarkers. These host genetic markers have been implemented in a diagnostic assay. It will potentially enable a more accurate diagnosis of women with CT associated subfertility.

In this keynote lecture, the state of art of host genetic markers in the susceptibility to and severity of chlamydial infection will be discussed with a main focus on CT infections and the potential role of zoonotic and environmental Chlamydiae and *Chlamydia*-like.

ORAL PRESENTATIONS

The genetic transformation of *Chlamydia pneumoniae*

Kensuke Shima[1]; Maximilian Wanker[1,2]; Rachel J. Skilton[3]; Lesley T. Cutcliffe[3]; Christiane Schnee[4]; Thomas A. Kohl[2,5]; Stefan Niemann[2,5]; Javier Geijo[6]; Matthias Klinger[7]; Peter Timms[8]; Thomas Rattei[6]; Konrad Sachse[9]; Ian N. Clarke[3]; Jan Rupp[1,2]

[1] Department of Infectious Diseases and Microbiology, University of Luebeck, Luebeck, Germany
[2] German Center for Infection Research (DZIF), partner site Hamburg-Luebeck-Borstel, Germany
[3] Molecular Microbiology Group, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, UK
[4] Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Jena, Germany
[5] Molecular and Experimental Mycobacteriology, Forschungszentrum Borstel, Borstel, Germany
[6] Division of Computational Systems Biology, University Vienna, Austria
[7] Institute of Anatomy, University of Luebeck, Luebeck, Germany
[8] University of Sunshine Coast, Maroochydore, Australia
[9] RNA Bioinformatics and High-Throughput Analysis, Faculty of Mathematics and Computer Science, Friedrich-Schiller-Universität Jena, Jena, Germany

Chlamydia pneumoniae infect animals and humans causing a wide range of different diseases. The absence of tools for the genetic manipulation of *C. pneumoniae* has severely hampered research into all aspects of its biology. Here, we demonstrate the genetic transformation of *C. pneumoniae* using a plasmid shuttle vector system which generates stable transformants.

The equine *C. pneumoniae* N16 isolate harbors the 7.5 kb plasmid pCpnEl. We constructed the plasmid vector pRSGFPCAT-Cpn containing a pCpnEl backbone, the red-shifted green fluorescent protein (RSGFP), as well as the chloramphenicol acetyltransferase (CAT) gene used for the selection of plasmid shuttle vector-bearing *C. pneumoniae* transformants. Using the pRSGFPCAT-Cpn plasmid-construct, expression of RSGFP in animal isolate of *C. pneumoniae* LPCoLN was demonstrated.

In previous studies it was shown that *Chlamydia* spp. cannot be transformed when the plasmid shuttle vector is constructed from a different plasmid backbone to the homologous species. Accordingly, we confirmed that pRSGFPCAT-Cpn could not cross the species barrier in plasmid-bearing and plasmid-free *C. trachomatis*, *C. muridarum*, *C. caviae*, *C. pecorum* and *C. abortus*. However, contrary to our expectation, pRSGFPCAT-Cpn did transform *C. felis*. Furthermore, pRSGFPCAT-Cpn did not recombine with the wild type plasmid of *C. felis*.

We provide for the first time an easy-to-handle transformation protocol for *C. pneumoniae* that results in stable transformants. The novel vector and technology

offer a promising new approach to investigate gene function and to study all aspects of *C. pneumoniae* biology. In addition, the vector can cross the species barrier to *C. felis*, indicating the potential of horizontal pathogenic gene transfer via a plasmid.

The role of polymorphic membrane proteins (Pmps) in *Chlamydia psittaci* strains with different infectious potential

Favaroni, A.; Schnee, C.

Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Jena, Germany

Chlamydia (*C.*) *psittaci* is the etiological agent for chlamydiosis in birds and can be transferred to humans by inhalation, causing severe systemic disease and pneumonia. *C. psittaci* infects a broader range of hosts and tissues and replicates faster than other *Chlamydiae*. Moreover, its infectious and zoonotic potential varies among avian and non-avian strains.

A comparative analysis of the genome of *C. psittaci* with the genomes of other chlamydial species revealed the regions of highest variability to be the plasticity zones and the coding sequences of Pmps. Several Pmps from different chlamydial species have been identified as immunoreactive proteins, recognized by specific antibodies present in human and animal sera; moreover, Pmps from the human pathogens *C. trachomatis* and *C. pneumoniae* are adhesins, relevant for the infection in a species-specific manner. Pmp genes and proteins are highly heterogeneous, suggesting a correlation with tissue tropism and pathogenicity. *C. psittaci* harbors 21 Pmps, heterogeneously expressed during the infection. Since Pmps are thought to be key players in chlamydial infection, the purpose of this project is to investigate whether the different zoonotic potential and host tropism of avian and non-avian *C. psittaci* strains may be dependent on a different set of Pmp expression.

To identify Pmps relevant for the *C. psittaci* infection, recombinant PmpB and PmpD of a non-avian isolate (DC15) have been produced. PmpB is the most conserved Pmp among different *Chlamydia* species, while PmpD is immunoreactive in several *Chlamydiae*. When analyzed for their functional properties, both PmpB and PmpD of *C. psittaci* were able to adhere with human and animal epithelial cells and could block a subsequent *C. psittaci* infection *in vitro*, showing their relevance for the infection. Bioinformatic analyses of the 21 Pmps of avian and non-avian *C. psittaci* isolates from different animal origins confirmed a high degree of variations of *pmp* genes, but this variation does not seem to be dependent on genotype or host. Thus, comparisons of Pmp expression profiles over the course of the infection of 10 *C. psittaci* strains from different genotypes and animal origin are being performed via RT-qPCR and via immunofluorescence, using antibodies raised against recombinant Pmps from *C. psittaci* DC15.

Analyses of Pmp expression profiles among different *C. psittaci* isolates might lead us to identify which Pmps may play a crucial role for their virulence and zoonotic potential.

Binding of elementary bodies the opportunistic fungal pathogen *Candida albicans* inhibits *Chlamydia trachomatis* infectivity

Michael Kruppa[1,2]; Jeremy Jacobs; Kelsey King-Hook; Amy Berry[1,2]; Jennifer Kintner[1,2]; Judy D. Whittimore[1,2]; Rolf Fritz[1,2]; Robert V. Schoborg[1,2]; Jennifer V. Hall[1,2]

[1] Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, TN
[2] Center for Infectious Disease, Inflammation and Immunity, Quillen College of Medicine, East Tennessee State University, Johnson City, TN

Interactions between pathogenic and/or commensal microbes represent an understudied facet of human health and disease. In this study, the interactions that occur between *Chlamydia trachomatis* and the opportunistic fungal pathogen, *Candida albicans* were investigated. *Candida albicans* is a common component of the oral and vaginal microbiota responsible for thrush and vaginal yeast infections. Normally, *Candida* exist in the body as yeast. However, disruptions to the microbiota, by antibiotics for example, create conditions that allow expanded growth of *Candida*, conversion to the hyphal form, and potential tissue invasion. Previous studies have shown that binding of *Staphylococcus* to *Candida* hyphae promotes dissemination of the bacteria into the blood stream of mice. Other studies suggest that *Candida* serves as a protective reservoir for the pathogen, *Helicobacter pylori*. To determine if *C. trachomatis* interacts with *C. albicans*, we incubated chlamydial elementary bodies (EB) in medium alone or with *C. albicans* yeast or hyphal forms for 1h. Following incubation, the samples were formaldehyde-fixed and processed for immunofluorescence assays using anti-chlamydial MOMP or anti-chlamydial LPS antibodies. Replicate samples were replenished with culture medium and incubated at 35 °C for 0–120 h prior to fixation for immunofluorescence analysis or collection for EB infectivity assays. Data from this study indicates that both *C. trachomatis* serovar E and *C. muridarum* EB bind to *C. albicans* yeast and hyphal forms. This interaction was not blocked by pre-incubation of EB with *Candida* cell wall components, mannan or beta-glucans, suggesting that EB interact with a *Candida* cell wall protein or other structure. Bound EB remained attached to *C. albicans* for a minimum of 5 days (120 h). Infectivity assays demonstrated that EB bound to *C. albicans* are infectious immediately following binding (0 h). However, once bound to *C. albicans,* EB infectivity decreased at a faster rate than EB in medium alone. At 6 h post binding, 40% of EB incubated in medium alone remained infectious compared to only 16% of EB bound to *C. albicans*. Likewise, pre-incubation of EB with *Candida* cell wall components alone or in combination significantly decreases chlamydial infectivity in

HeLa cells. These data indicate that interactions of EB with *C. albicans* inhibits chlamydial infectivity, possibly by physically blocking EB interactions with host cell receptors or secretion of inhibitory compounds by *Candida albicans*.

Characterization of cell division mechanisms of *Waddlia chondrophila*, a *Chlamydia*-related bacterium

Bayramova, F.; Jacquier, N.; Greub, G.

Institute of Microbiology, Lausanne University Hospital and Lausanne University, Rue du Bugnon 48, CH-1011 Lausanne, Lausanne, Switzerland

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* differs from the classical division machinery by lacking the homologue of the bacterial division organizer FtsZ and certain division proteins homologues. Nevertheless, *Chlamydiales* generally divide by binary fission. Another specificity of the *Chlamydiales* order is an atypical peptidoglycan, which might have an important role in chlamydial division. We recently described septal proteins, MreB, RodZ and NlpD, which play important roles in *W. chondrophila* cell division and in peptidoglycan remodelling. Actin homologue MreB and its regulator RodZ have been localized at the chlamydial division septum. It was shown that MreB inhibitors can block chlamydial proliferation and that the NlpD enzyme is also able to localize at the division septum and remodel peptidoglycan. In this work, we aim to characterize the composition of the chlamydial division machinery, describe the exact role of the septum proteins and their interactors involved in chlamydial divisome formation and peptidoglycan remodeling.

In order to get a complete picture of the chlamydial divisome composition, potential interactors of the described septal protein RodZ were detected using Yeast-two-hybrid assay and co-immunoprecipitation. Moreover, proteins binding to peptidoglycan were analysed by mass spectrometry. Comparing these potential interactors with what was known on them in other bacteria, we selected SecA, FtsH and SufD proteins for further studies. We first investigated the pattern of gene expression of these potential division proteins by qRT-PCR. Expression pattern of these genes were consistent with a potential role in chlamydial division. We then examined the localization of SecA, FtsH and SufD proteins in proliferating *W. chondrophila* by immunofluorescence. Moreover, we also observed the localization of these proteins in enlarged bacteria called aberrant bodies that were induced by treatment of *W. chondrophila* with peptidoglycan synthesis inhibitors such as penicillin and phosphomycin. We could observe a peripheral localization of these proteins, consistent with their putative colocalization with RodZ, which resides in the inner membrane. Interestingly, localization of SecA and FtsH after penicillin treatment is reminiscent of the accumulation of RodZ at aborted division septa after penicillin treatment.

Furthermore, to confirm the involvement of putative interactors in bacteria division, the above-mentioned proteins from *W. chondrophila* were heterologously overexpressed in *E. coli*. Overexpression of the proteins in *E. coli* in stringent conditions revealed an impaired bacterial growth and partial proliferation inhibition compared to the controls. The effect of this overexpression on *E. coli* morphology was assessed by microscopy. We could observe that overexpression of the above-mentioned proteins in *E. coli* leads to a more prolonged and enlarged shape compared to the rod-shaped control group.

Nowadays chlamydial division mechanism is still poorly understood. By using Y2H assay, potential interactors of the division septum protein RodZ were identified. *secA*, *ftsH* and *sufD* transcripts could be detected at early times points during the *W. chondrophila* developmental cycle, which is consistent with a role of these genes in chlamydial division.

SecA is part of the secretion apparatus and was shown to be localized at the division septum in other bacteria. We could observe here, that it might be the case also in *Chlamydiae*. This is of particular interest since many proteins, such as peptidoglycan modifying enzymes and peptidoglycan-binding proteins need to be translocated to the periplasm during division.

FtsH is a protease that was localized at the division septum of *Bacillus subtilis*. It might thus play a potential role in activation and/or degradation of septal proteins during chlamydial division. This is consistent with its localization to aborted septa in *W. chondrophila*.

Finally, SufD might bring a link between division regulation and iron deprivation. Indeed, upon iron deprivation, chlamydial division is inhibited by an unknown mechanism that causes the formation of aberrant bodies. We could not observe a clear septal localization of SufD, indicating that interaction of SufD with RodZ might be transient or could happen in very specific conditions.

Interestingly, heterologous overexpression of these proteins in *E. coli* caused morphology defects and induced a slower proliferation, comforting the potential role of these proteins in bacterial division.

Nevertheless, much has to be done to understand the exact role these proteins play in chlamydial division. This might lead to the discovery of new potential drug targets allowing inhibition of chlamydial proliferation.

The influence of centrifugation and incubation temperature on various veterinary and human chlamydial species

Onorini, D.[2]; Donati, M.[2]; Marti, H.[1]; Biondi, R.[2]; Levi, A.[2]; Nufer, L.[1]; Rigamonti, S.[3]; Vicari, N.[3]; Borel, N.[1]

[1] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland
[2] DIMES, Microbiology, Policlinico S. Orsola, University of Bologna, Italy
[3] National Reference Laboratory for Animal Chlamydioses, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Pavia, Italy

The *Chlamydiaceae* are a family of Gram-negative bacteria causing various diseases in humans and in both endothermic (mammals and birds) and poikilothermic (e.g. reptiles, amphibians) animals (Borel et al., 2018; Cevenini et al., 2002). As most chlamydial species described today were isolated from humans and endothermic animals, the commonly used culturing temperature is at 37 °C, however, during experimental infection, centrifugation temperatures may vary (Staub et al., 2018; Suwarak et al., 2017). The aim of this study was to investigate the influence of centrifugation and incubation temperature on the ultrastructural morphology and infectivity of two human chlamydial strains, *C. trachomatis* serovar E and *C. pneumoniae* Kajaani 6, two known zoonotic chlamydial strains, *C. psittaci* 6BC and *C. abortus* S26/3, two well-described animal *Chlamydia* species, *C. suis* from pigs and *C. pecorum* from ruminants, as well as two recently described species originating from snakes, *C. poikilothermis* and *C. serpentis*.

Briefly, we performed single infections of LLC-MK2 cells (rhesus monkey kidney cells) with the chlamydial strains used in this study aiming for an infection rate of 30–40%. Post inoculation, centrifugation was performed either at 28 °C or 33 °C for 1 h at 1000 *g*. After centrifugation, inocula were replaced with fresh medium at their respective incubation temperature of either 28 °C or 37 °C resulting in three conditions per strain (28 °C centrifugation/28 °C incubation; 28 °C/37 °C; 33 °C/37 °C). After an incubation period of 48 h, cultures were either collected for titration by sub-passage to determine the infectivity per condition or fixed either in methanol for immunofluorescence assay (IFA) or fixed in glutaraldehyde for transmission electron microscopy (TEM).

IFA analysis consisted of the rough determination of the infection rate, a qualitative assessment of the inclusion morphology and a semi-quantitative analysis of the average inclusion size per strain and condition. TEM sections were screened for chlamydial inclusions. In 10 inclusions per strain and condition, the number of each chlamydial developmental form consisting of reticulate bodies (RBs), dividing RBs, intermediate bodies (IBs) and elementary bodies (EBs) was determined as described in other publications (Marti et al., 2014; Lee et al., 2018).

We found that the infectivity and average inclusion size was reduced at an incubation temperature of 28 °C compared to 37 °C for all strains including *C. poikilothermis*,

though the latter appeared to contain more infectious EBs at 28 °C incubation temperature than the other species. We further found that higher centrifugation temperatures promote the subsequent inclusion size of *C. suis*, *C. trachomatis* and *C. abortus* but not their infectivity. The incubation temperature had no discernable effect on the morphology, inclusion size and infectivity on the remaining chlamydial strains.

Immunohistochemical demonstration of the tissue tropism of *Chlamydia pecorum* in porcine abortions

Liebler-Tenorio, E. M.[1], Hansen, M. S.[2], Jensen, T. K.[2]

[1] Institute of Molecular Pathogenesis, Friedrich-Loeffler Institut, Jena, Germany
[2] National Veterinary Institute, Technical University of Denmark, Kgs. Lyngby, Denmark

Chlamydia pecorum was identified by fluorescent *in situ* hybridization (FISH) using species-specific probes in 38 of 138 cases (28%) of spontaneous porcine abortions. As *C. pecorum* is not a common cause of abortion in pigs, IHC was used to investigate distribution and potential association between chlamydial inclusions and tissue lesions.

Formalin-fixed paraffin-embedded placenta and fetal organs (lung, heart, liver, kidney, umbilical cord) of 15 FISH-positive cases were examined for chlamydial antigen using proteinase K for antigen retrieval and the indirect immunoperoxidase method. A negative control was included for each section.

Chlamydial inclusions were detected in 14 of 15 cases with necrotizing and sometimes purulent placentitis and purulent bronchopneumonia. Only one case with placentitis and necrotizing hepatitis, but no pulmonary lesions was negative. Chlamydial inclusions were found in fetal pulmonary tissue (in 13/15), in fetal placenta (in 10/12) and in the umbilical cord (in 9/14). Liver, kidney and heart of all fetuses were negative for chlamydial antigen. Labelling in the lungs was variable and predominantly associated with the cellular exudate in the airways. In addition, epithelial cells of bronchioles and rarely alveoli were positive. In the placenta, Chlamydial inclusions were present both in cellular detritus between chorionic villi, trophoblasts and allantoic epithelium. Chlamydial inclusions were also seen in the amniotic epithelium lining the umbilical cord of 9 fetuses and in epithelial cells of the urachus of 6 fetuses, but not associated with lesions in these tissues.

In conclusion, presence of chlamydia was confirmed by immunohiostochemistry. Chlamydial inclusions in placenta and fetal lungs were associated with placentitis and bronchopneumonia. Thus, infection with *C. pecorum* which is predominantly implicated in infections of ruminants should be included as differential diagnosis in porcine abortions.

Understanding tetracycline resistance in *Chlamydia suis*

Peisker, M.; Berens, C.; Schnee, C.

Institute of Molecular Pathogenesis, Friedrich-Loeffler Institut, Jena, Germany

The tetracycline resistance determinant TetC is the sole example of a genetically stable resistance acquired by an obligate intracellular bacterium by horizontal gene transfer. It is located on a genomic island in *Chlamydia* (*C.*) *suis*, a pathogen causing inapparent infections but also respiratory, intestinal and genital disorders in pigs. The TetC cassette harbors the resistance gene *tetC*, the repressor *tetR*(C) and a control region, and was shown to be in vitro-transferable from porcine *C. suis* to the closely related human pathogen *C. trachomatis* which could be of particular public health concern.

In an ongoing prevalence study, we have examined 200 pigs from 10 farms in Germany by real time PCR and found a sample prevalence of 45.3% and a farm prevalence of 100% for *C. suis*. In 93.0% of the *C. suis*-positively tested samples, the tetracycline resistance gene *tetC* was detected by PCR. In comparison, in 89 feral pigs sampled after hunting, the sample prevalence for *C. suis* was lower with 21.0%. Interestingly, *tetC* was detected in 47.2% of the positive samples indicating a spread of the tetracycline resistance determinant in wild boar populations even without direct selective pressure by antibiotic treatment.

Another aim of our study was to characterize and compare *C. suis* isolates regarding the genetic structure, regulation and functionality of their TetC resistance determinant. 16 selected isolates tested PCR-positive for the *tetC* resistance gene, but showed different degrees of susceptibility to tetracycline with MIC values varying from 1 µg/ml to 8 µg/ml. To elucidate these differences, we investigated the functionality of the control region and *tetC* in a heterologous setting. First, the control region activity of six isolates was analyzed in a plasmid-encoded reporter gene system in *Escherichia* (*E.*) *coli*. Despite containing several different point mutations, the control regions of all isolates were functional. In a second heterologous expression trial, *tetC* from the six *C. suis* strains were shown to refer resistance to the *E. coli* host, demonstrating functionality of the resistance gene in all cases. In conclusion, the genetic structure of the control region and *tetC* seems not to be responsible for the observed differences in tetracycline susceptibility in *C. suis*.

When we compared the effect of different tetracyclines, selected isolates showed high MIC values (4–32 µg/ml) with tetracycline and oxytetracycline applied in pig farming, but seemed susceptible to doxycycline and minozycline (MIC 0.125–1 µg/ml) used in human therapy. Thus, the opportunity of horizontal gene transfer of TetC to *C. trachomatis* might not be such a serious concern as considered before.

wIRA/VIS irradiation reduces chlamydial infectivity of *C. trachomatis* independent of targeted cytokine and chemokine inhibition

Kuratli, J.[1] ; Pesch, T.[1] ; Marti, H.[1] ; Blenn, C.[1] ;
Leonard, C.[1]; Torgerson, P.[2] ; Borel, N.[1]

[1] Institute of Veterinary Pathology Zurich (IVPZ), Vetsuisse Faculty,
University of Zurich, Winterthurerstrasse 268, 8057 Zurich
[2] Section of Veterinary Epidemiology, Vetsuisse Faculty,
University of Zurich, Winterthurerstrasse 270, 8057 Zurich

Chlamydia trachomatis is the major cause of infectious blindness and represents the most common bacterial sexually transmitted infection worldwide. Considering the potential side effects of antibiotic therapy and increasing threat of antibiotic resistance, alternative therapeutic strategies are needed.

Previous studies showed that water filtered infrared A alone (wIRA) or in combination with visible light (wIRA/VIS) reduced *C. trachomatis* infectivity. Furthermore, wIRA/VIS irradiation led to secretion of pro-inflammatory cytokines similar to that observed upon *C. trachomatis* infection.

We confirmed the results of previous studies, namely that cytokine secretion (IL-6, IL-8, RANTES) upon wIRA/VIS treatment, and the subsequent reduction of chlamydial infectivity, are independent of the addition of cycloheximide, a host protein synthesis inhibitor. Constant, reproducible cytokine release upon irradiation led us to hypothesize that cytokines might be involved in the anti-chlamydial mechanism of wIRA/VIS. We tested this hypothesis by inhibiting IL-6, IL-8 and RANTES secretion in *C. trachomatis* infected or mock infected cells by gene silencing or pharmacological inhibition. Celastrol, a natural substance derived from *Trypterygium wilfordii*, used in traditional Chinese medicine and known for anti-cancer and anti-inflammatory effects, was used for IL-6 and IL-8 inhibition, while Maraviroc, a competitive CCR5 antagonist and anti-HIV drug, served as a RANTES/CCL5 inhibitor.

HeLa cell cytotoxicity and impact on chlamydial morphology, size and inclusion number was evaluated upon increasing inhibitor concentration, and concentrations of 0.1 and 1 µM Celastrol and 10 and 20 µM Maraviroc were subsequently selected for irradiation experiments. Celastrol at any concentration reduced chlamydial infectivity, an effect only observed for 20 µM Maraviroc. Triple dose irradiation (24, 36, 40 hpi) significantly reduced chlamydial infectivity in HeLa cells regardless of IL-6, IL-8 or RANTES gene silencing, Celastrol or Maraviroc treatment. Neither gene silencing nor pharmacological cytokine inhibition provoked the chlamydial stress response.

Thus, the anti-chlamydial effect of wIRA/VIS was independent of cytokine inhibition under all conditions evaluated. This study gives first insight into the mechanism of wIRA/VIS in relation to an integral component of the host immune system and supports the potential of wIRA/VIS as a promising new tool for treatment of trachoma.

What is the importance of abortions due to the live vaccine *C. abortus* 1B strain in sheep flocks?

Laroucau, K.[1]; Soubrier, T.[2], Aaziz, R.[1]; Vorimore, F.[1]; Longbottom, D.[3]; Pouget, C.[4]; de Cremoux, R.[5]

[1] University Paris-Est, ANSES, Animal Health Laboratory, Bacterial Zoonoses Unit. Maisons-Alfort, France
[2] National Veterinary School, Toulouse, France
[3] Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Midlothian, EH26 0PZ, United Kingdom
[4] GDS12, Rodez, France
[5] French Livestock Institute, Mixt Technological Unit on Small Ruminants Health, Albi, France

Chlamydia abortus is responsible for enzootic abortion in both sheep and goats and has major economic implications for the farming industry worldwide. A virulence-attenuated mutant strain of C. abortus (strain 1B) is currently commercially available as a live attenuated vaccine for immunization of sheep and goats in several European countries. Recent studies have suggested a link between the vaccine strain and disease.

Here we document an abortion outbreak involving the vaccine strain in a flock of 200 ewes, which exhibits similar clinical expression to a natural infection with a wild type strain and report the results of a larger survey conducted in the South of France on sheep flocks that reported series of abortions in order to describe the risk factors potentially associated with a chlamydiosis occurrence according to the vaccination context.

Development of a new vaccine to protect sheep from ovine enzootic abortion

Livingstone, M.; Aitchison, K.; Wattegedera, S.; Rocchi, M.; Wilson, K.; Sait, M.; Wheelhouse, N.; Chianini, F.; Entrican, G.; Longbottom, D.

Moredun Research Institute, Pentlands Science Park, Edinburgh, United Kingdom

Chlamydia (*C.*) *abortus*, the aetiological agent of Ovine Enzootic Abortion (OEA), is a major cause of reproductive loss in small ruminants throughout the world and is consistently the most common diagnosed cause of abortion in the UK thereby accounting for significant economic losses to the farming industry. Disease management in the UK is achieved through the use of commercially available inactivated whole organism (Mydiavac) or live attenuated (Enzovax or Cevac Chlamydia) vaccines. Each vaccine type has its merits and limitations. In particular, there are general safety concerns over the live vaccines, which have been implicated in clinical cases of OEA [1–3]. Furthermore, recent whole genome analysis of the temperature-sensitive attenuated vaccine strain 1B and its parent strain AB7 has shown that there is no genetic evidence of any attenuation [4]. The aim of this study was to

evaluate the protective efficacy of subcellular and recombinant antigens as safer, cheaper and more stable alternative vaccines against OEA.

Scotch Mule sheep (aged 3 to 5 years), seronegative for *C. abortus* were randomly assigned to 6 groups. Groups 1 to 4 were vaccinated with the commercially available live-attenuated vaccine Cevac® Chlamydia (Group 1), two subunit vaccines (Groups 2 and 3) and a cocktail of recombinant antigens (Group 4). All vaccines were adjuvanted with Montanide ISA 70VG (Seppic), with the exception of Cevac® Chlamydia, and administered intramuscularly. An additional two groups served as positive and negative controls and were not vaccinated (Groups 5 and 6, respectively). At 70 days of gestation all vaccinated ewes and group 5 positive control ewes were challenged with *C. abortus*. The clinical outcome was recorded for each ewe and samples collected for pathological, microbiological, molecular and serological examination. The results of the trial will be presented and discussed.

References

1. Wheelhouse et al. 2010. Vaccine 28, 5657-5663.
2. Livingstone et al. 2014. Vet Record 174, 613-614.
3. Sargison et al. 2015. New Zealand Veterinary Journal 63, 284-287.
4. Longbottom et al. 2018. Vaccine 36, 3593–3598.

Effect of progesterone on the vaccination and immune response against *Chlamydia abortus* in the natural host

A. Murcia-Belmonte[1]; D. Álvarez[1]; N. Ortega[1]; J. A. Navarro[2]; E. Gómez-Lucía[3]; A. J. Buendía[2]; L. Del Rio[1]; J. Salinas[1]; M. R. Caro[1]

[1] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, España
[2] Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Murcia, España
[3] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, España

Chlamydia abortus is the causative agent of the ovine enzootic abortion (OEA). Infection remains subclinical until ewes becomes pregnant, when reactivation of infection occurs, leading to pathological processes and eventually, abortions. After this, animals acquire protective immunity. However, they become carriers and shedding of the organism in following oestrus cycles. This process of latency/active multiplication that leads to the recrudescence of *C. abortus* suggests that female sex hormones might play an important role on the physiopathology of the OEA that could affect the success of a chlamydial clearance and also jeopardising the effectiveness of a vaccination. However, the mechanisms by which sex hormones are involved in chlamydial pathogenicity remain unclear. Therefore, the aim of this study was to determine the

effect of progesterone on the immune response against *C. abortus* and on the protection conferred by an experimental inactivated vaccine in sheep.

Eighteen sheep were ovariectomised and allocated into 4 groups: vaccinated and progesterone-treated (V-PG), vaccinated and non-treated (V-NT), non-vaccinated and non-treated (NV-NT) and non-vaccinated and progesterone-treated sheep (NV-PG). Animals from PG groups were treated with commercial medroxi-progesterone acetate impregnated intravaginal sponges, before and during the vaccination (V-PG) or just before challenge (NV-PG). On the other hand, animals from V groups were subcutaneously immunized with an experimental inactivated vaccine, which proved to confer high protection in previous studies. All sheep were challenged intratracheally with 5 x 10^7 inclusion-forming units of *C. abortus* strain AB7 and they were sacrificed at day 8 post-infection. The morbidity was measured as the variation of the rectal temperature and samples of sera were collected for antibodies and cytokines (IFN-γ and IL-10) analyses by commercial ELISA. In addition, lung and lymph nodes samples were collected for chlamydial detection by qPCR and for histopathological and immunohistochemical analyses.

Sheep from V-PG group showed less severe lesions and morbidity than other groups. Besides they had the highest antibodies level against *C. abortus*. On the other hand, sheep from V-NT showed also a high antibody levels against *C. abortus* and less severe lesions than observed in non-vaccinated sheep (NV-NT and NV-PG) which showed high morbidity, low antibody levels and severe lesions, especially in NV-NT. These results support the effectiveness of the experimental vaccine employed and suggest that progesterone could enhance it. This could mean that the best time for sheep immunization against *C. abortus* is when progesterone levels are highest during the oestrus cycle.

Study on an inactivated vaccine against *Chlamydia abortus* isolated from yaks (*Bos grunniens*) and immunization tests in yaks

Zhao, R.[1]; Kan, W.[1,2]; Li, Z. C.[1]; Cai, J. S.[2]; Lou, Z. Z.[1];
Chao, Y. L.[2]; Fei, Y. Y.[1]; Zhang, Z. J.[1]; Zhou, J. Z.[1]

[1] State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 730046 Lanzhou City, Gansu Province, PR China
[2] Qinghai Animal Disease Control Center, 810000 Xining City, Qinghai Province, PR China

The GN-6 strain of *Chlamydia abortus* (*C. abortus*) isolated from yaks (*Bos grunniens*) was used to prepare an inactivated vaccine by means of proliferation in chicken embryos, inactivation, and emulsification, etc. Then mice were vaccinated with the prepared vaccine and their serum was collected at 21 days after immunization for the following experiments: detection of immune antibodies, isotype identification of serum

antibody, measurement of IFN-γ level, detection of neutralizing antibodies and delayed-type hypersensitivity (DTH), etc. A viral challenge test and a lymphocyte proliferation test were also performed in vaccinated mice. The results showed: Not only that the vaccinated mice produced neutralizing antibodies, but also serum antibodies produced were of IgG1 type, and a certain amount of IFN-γ was also present in the serum, suggesting that the vaccinated mice had developed a significant Th1-type immune response. Results of DTH test showed thickened footpads in the control mice, which suggested the presence of a specific cellular immune response. The challenge test showed the experimental mice were protected by the studied vaccine while the control mice were infected by the pathogens. A total of 35 newly weaned yaks (2–4 months old) and 35 pregnant adult yaks were used for immunoprotection experiments. The results of this study showed a protection rate of 100% in the experimental yaks without a single case of abortion compared to an abortion rate of 73.3% in the control yaks, indicating that the investigational inactivated vaccine can protect yaks from the attack of *C. abortus*.

Key words: yaks; *Chlamydia abortus*; inactivated vaccine; mice; immunization.

Development of *Chlamydia pecorum* induced arthritis in lambs

Md. Mominul Islam[1]; Martina Jelocnik[1]; Narelle Sales[2]; Ian Marsh[2]; Mark Hazelton[2]; Sam Gilchrist[2]; Nicole Borel[3]; Adam Polkinghorne[1]

[1] Animal Research Centre, University of the Sunshine Coast, Sippy Downs, Australia
[2] Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia
[3] Institute of Veterinary Pathology, University of Zurich, Switzerland

Chlamydia (C.) pecorum is the causal agent of a range of infectious diseases in sheep, cattle and goats. The most economically significant of these diseases is polyarthritis. Molecular evidence suggests that genetically distinct strains of *C. pecorum* have different pathogenic potentials. Experimental evidence of this relationship is lacking, however, and the pathogenesis of *C. pecorum*-associated polyarthritis otherwise remains unknown. This study examined the ability of two *C. pecorum* strains isolated from the joint of a sheep with polyarthritis (IPA) and the brain of a calf with sporadic bovine encephalomyelitis (E58) to induce arthritis in 5–6 month old lambs. Animals (n=20) were divided evenly into IPA and E58 infection group and received 10^7 inclusion forming units (IFU) of *C. pecorum* either via intra-articular (IA; n = 5 per strain) or intravenous route (IV; n = 5 per strain). A control group (n = 15) received either UV inactivated *C. pecorum* via IA or IV inoculation or Sucrose Phosphate Glutamate (SPG) by IA. As expected, all IA-inoculated sheep (10/10) infected with viable IPA or E58 strains developed lameness within 24–48 hr post-infection (PI). Only three animals (3/5; 60%) from the IPA IV administration group developed lameness, 7–9 days PI, which eventually resolved after 3–5 days. No evidence of lameness was observed in the E58 IV group or in any control animal. IPA group developed more prominent and severe joint pathology than E58 groups. The preliminary results of this

work suggest that *Chlamydia*-associated arthritis can be induced experimentally and that key differences may indeed exist in the pathogenic potential of certain C. *pecorum* strains. Further work is now underway to assess bacterial shedding pattern and further pathological differences between infection cohorts. This infection model will be useful to understand the factors influencing chlamydial arthritis in sheep and serve as a valuable tool in the development and evaluation of novel control strategies for this widespread livestock pathogen.

***Waddlia chondrophila*: from bovine abortion to human miscarriage**

Ammerdorffer, Anne[1,2]; Dooremalen van, Wies[1,2]; Morré, Servaas A.[1,2,3]

[1] TubaScan Ltd, de Boelelaan 1108, 1081 HZ Amsterdam, Netherlands
[2] Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Medical Microbiology and Infection Control, Laboratory of Immunogenetics, De Boelelaan 1108, 1081 HZ Amsterdam, Netherlands
[3] Institute for Public Health Genomics, Department of Genetics and Cell Biology, Research Institute GROW, Faculty of Health, Medicine & Life Sciences, University of Maastricht, Maastricht, The Netherlands

In contrast to the familiar Chlamydiales *C. trachomatis* and *C. abortus*, its relatives *Simkania negevensis*, *Parachlamydia acanthamoebae* and *Waddlia chondrophila* are less well described.

W. chondrophila was first reported in 1990 when a new obligate intracellular organism was isolated from lung and liver samples of an aborted bovine fetus [1]. In 2002, a second case of *W. chondrophila* was found in a septic stillborn calf [2]. Moreover, when two bovine fetuses were experimentally infected with *W. chondrophila*, one fetus died within 2 weeks [3]. In contrast, a more recent publication from Wheelhouse *et al.* [4] did not confirm the abortifacient potential of *W. chondrophila* after experimental infection of cattle, although they did detect *W. chondrophila* in one of the infected placentas.

W. chondrophila is a zoonotic bacterium, and several studies identified *W. chondrophila* to be associated with adverse pregnancy outcomes and infertility problems such as tubal factor infertility (TFI) in women [5–8]. Our group published a study in which serum samples from women with and without TFI were tested for *W. chondrophila* antibodies. This revealed a high prevalence in our study population (520 women) for *W. chondrophila* (45.5%), and high titers of *W. chondrophila* antibodies are associated with TFI, independent of *C. trachomatis* [7]. A study performed by Baud *et al.* showed a *W. chondrophila* seroprevalence of 33% in a group of English women suffering from recurrent miscarriages [6]. *In vitro* studies have demonstrated the ability of *W. chondrophila* to infect a variety of cell lines, including endometrial cells supporting its role in subfertility [9].

There are several potential risk factors for human infection by *W. chondrophila*. It includes among others milk consumption, eating uncooked meat and contact with animals. Another possible source for infection can be (drinking) water, as free-living amoebae present in water are permissive to *W. chondrophila* infection. Two separate studies in Spain and France show the presence of *W. chondrophila* DNA in respectively well water and hot water systems [10, 11]. Our unpublished data also reveal a low presence of *W. chondrophila* DNA in two of the 11 water stations tested.

So far, the work on *W. chondrophila* is limited and sometimes the findings are contradictory. However, its potential adverse effect on reproduction and its high seroprevalence encourages further research.

References

1. Dilbeck, P.M., et al., *Isolation of a previously undescribed rickettsia from an aborted bovine fetus.* J Clin Microbiol, 1990. **28**(4): p. 814-6.
2. Henning, K., et al., *Neospora caninum and Waddlia chondrophila strain 2032/99 in a septic stillborn calf.* Vet Microbiol, 2002. **85**(3): p. 285-92.
3. Dilbeck-Robertson, P., et al., *Results of a new serologic test suggest an association of Waddlia chondrophila with bovine abortion.* J Vet Diagn Invest, 2003. **15**(6): p. 568-9.
4. Wheelhouse, N., et al., *Experimental challenge of pregnant cattle with the putative abortifacient Waddlia chondrophila.* Sci Rep, 2016. **6**: p. 37150.
5. Baud, D., et al., *Role of Waddlia chondrophila placental infection in miscarriage.* Emerg Infect Dis, 2014. **20**(3): p. 460-4.
6. Baud, D., et al., *Waddlia chondrophila, a potential agent of human fetal death.* Emerg Infect Dis, 2007. **13**(8): p. 1239-43.
7. Verweij, S.P., et al., *Waddlia chondrophila and Chlamydia trachomatis antibodies in screening infertile women for tubal pathology.* Microbes Infect, 2015. **17**(11-12): p. 745-8.
8. Ammerdorffer, A., et al., *Chlamydia trachomatis and chlamydia-like bacteria: new enemies of human pregnancies.* Curr Opin Infect Dis, 2017. **30**(3): p. 289-296.
9. Kebbi-Beghdadi, C., O. Cisse, and G. Greub, *Permissivity of Vero cells, human pneumocytes and human endometrial cells to Waddlia chondrophila.* Microbes Infect, 2011. **13**(6): p. 566-74.
10. Codony, F., et al., *Well water as a possible source of Waddlia chondrophila infections.* Microbes Environ, 2012. **27**(4): p. 529-32.
11. Agusti, G., et al., *Presence of Waddlia chondrophila in hot water systems from non-domestic buildings in France.* J Water Health, 2018. **16**(1): p. 44-48.

Feed-borne *Bacillus cereus* aggravates respiratory distress of *Chlamydia psittaci*

Zuo, Z. H.[1,2]; Li, Q.[1]; Zhang, T. Y.[1]; Zhang, Q.[1];
Guo, X. G.[1]; Hegemann, J. H.[2]; He, C.[1]

[1] Key Lab of Animal Epidemiology and Zoonosis, College of Veterinary Medicine, China Agricultural University, Beijing 100193, PR China
[2] Department of Biology, Institute for Functional Microbial Genomics, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, 40204, Germany

Chlamydia psittaci (C. psittaci) can cause ornithosis and is associated with air sacculitis in poultry farms of China. Although it is recognized as a respiratory pathogen, the oral route still plays an important role in chlamydial transmission and persistent infection. *Bacillus cereus* (*B. cereus*) is widely used as probiotics in animal feed globally. Recently, we have isolated a *B. cereus* strain (Dawu C) from the chicken feed which harbored two virulence genes *(hblC and cytk*). This isolate was able to induce a gizzard erosion and ulceration (GEU) syndrome in broilers. In this study, we tested whether a *B. cereus*-induced GEU might have an impact on the oral infection of *C. psittaci* in chickens.

80 SPF chickens (7 days old) were randomly distributed into 4 groups. Two groups were daily intraesophageally inoculated with *B. cereus* (Dawu C). The other two groups were treated the same way with PBS. At day 14, one *B. cereus* treated group and one PBS treated group were infected with *C. psittaci* intranasally and the other two groups were infected intraesophageally. Necropsy revealed that chickens administrated with *B. cereus* developed a severe GEU syndrome. Birds with GEU syndrome and intraesophageally infected with *C. psittaci*, over time developed significantly elevated chlamydial loads in the lung. Lesions in respiratory organs also developed to a similar level in *C. psittaci*-intranasally infected birds. At day 42, birds inoculated with *B. cereus* showed a remarkably decreased *C. psittaci*-specific proliferation of peripheral blood lymphocytes and an increase of IL-6 and IL-10 in lung lavage fluids. However, there was no significant difference in serum IgG level between *C. psittaci* infected and non-infected birds. Thus, *B. cereus* (Dawu C) aggravated oral infection of *C. psittaci* and could finally impair respiratory organs as well. Moreover, GEU caused by *B. cereus* facilitated chlamydial transmission from the stomach to the lung. In conclusion control of food-borne *B. cereus* is urgently required once *C. psittaci* is discovered in poultry farms.

Keywords: *Bacillus cereus, Chlamydia psittaci,* Gizzard erosion and ulceration (GEU), Respiratory distress, Chickens

Chlamydiales and hemotropic mycoplasmas in captive and free-living bats in Switzerland, Germany and Costa Rica

Fritschi, J.[1]; Aeby, S.[2]; Greub, G.[2]; Pilo, P.[3]; Mühldorfer, K.[4]; Stokar-Regenscheit, N.[5]; Wiederkehr, D.[6]; Bijleveld, C.[7]; Borel, N.[1]

[1] Institute of Veterinary Pathology, Vetsuisse-Faculty, University of Zurich, Switzerland
[2] Institute of Microbiology, University of Lausanne, Switzerland
[3] Institute of Veterinary Bacteriology, Vetsuisse-Faculty, University of Bern, Switzerland
[4] Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany
[5] Institute of Animal Pathology, Vetsuisse-Faculty, University of Bern, Switzerland
[6] Bern University of Applied Sciences, School of Agricultural, Forest and Food Sciences, Zollikofen, Switzerland
[7] Stiftung Papiliorama, Kerzers, Switzerland

Bats are susceptible to a variety of microorganisms, some of which are known as pathogens in humans and pets. However, little is known about the occurrence of *Chlamydiales* and hemotropic mycoplasmas in bats. In 2005 and 2015, two novel *Chlamydia*-like organisms (CLOs), *Waddlia malaysiensis* and *Waddlia cocoyoc* have been detected in fruit bats in Malaysia and Mexico, respectively (Chua et al. 2005; Pierle et al. 2015). In 2016, members of the order *Chlamydiales* were detected in the fecal bacterial flora of Daubenton's bats in Finland (Hokynar et al. 2016). Besides these findings, there are no published reports on the occurrence of *Chlamydiales* in bats to this date. This indicates a great potential for novel findings concerning the occurrence of *Chlamydiaceae* and CLOs in bats. Between 2014 and 2017, four projects to determine the prevalence of hemotropic mycoplasmas in various bat species were carried out (Ikeda et al. 2017, Mascarelli et al. 2014, Millán et al. 2015, Volokhov et al. 2017). The prevalence varied from 18.5% in the study from Brazil (Ikeda et al. 2017) to 96.8% in the study from Spain (Millán et al. 2014). In all studies, sequences obtained from the positive samples indicated the presence of a new species or genotype.

In this study, 89 captive bats from a Swiss zoo, 285 free-living bats from all across Switzerland, 28 captive and 56 free-living bats from Germany and 17 free-living bats from Costa Rica were investigated for the occurrence of *Chlamydiales* and hemotropic mycoplasmas.

The captive bats from Switzerland were necropsied and samples of selected organs were taken, whereas the samples of the free-living bats from Switzerland were obtained either as FFPE blocks or fixed in 4% formalin. DNA from all samples was extracted using either the DNeasy Blood and Tissue Kit (Qiagen) or the QIAamp DNA FFPE Tissue Kit (Qiagen). Captive and free-living bats from Germany (Mühldorfer et al. 2011a,b) or from Costa Rica were necropsied and homogenates of selected organs were used for DNA extraction (Macherey-Nagel). Initial screening for *Chlamydiales* was performed using the *Chlamydiaceae*-specific real-time PCR targeting the 23S rRNA

gene (Ehricht et al. 2006) which resulted in negative samples only. A second screening was performed using the pan-*Chlamydiales* PCR targeting the 16S rRNA gene (Lienard et al. 2011), in which *Chlamydiales* DNA was detected in 48/117 (41%) captive and 7/358 (2%) free-ranging bats. For the sequencing of the positive samples, a PCR was performed with the specifically designed inner primers panFseq and panRseq. A total of 19 valid sequences was obtained and classification criteria published by Pillonel et al. (2015) were used to classify these 19 sequences. 16/19 (84.2%) sequences are considered to be new family lineages in the order *Chlamydiales*, 1/19 sequences (5.3%) belonged to the same family as the uncultured bacterium clone 16S(V3-V4)-3471 (*Parachlamydiaceae*) and 1/19 sequences (5.3%) belonged to the same family as the uncultured bacterium clone LT100PlH9 (*Parachlamydiaceae*). Analysis for the presence of hemotropic mycoplasmas was performed using a conventional PCR targeting a 165 bp region of the RNaseP gene (Maggi et al. 2013) with minor modifications. DNA of hemotropic mycoplasmas was detected in 7/117 (6%) captive and 12/358 (3.4%) free-ranging bats. CLOs and hemotropic mycoplasmas are present in captive and free-ranging bats from Europe (Switzerland, Germany) and Costa Rica. However, their impact on bat's health is unknown to date.

An update on *Chlamydiaceae* in wild, feral and domesticated pigeons in Switzerland

Mattmann, P.[1]; Marti, H.[2]; Borel, N.[2]; Albini, S.[1]; Vogler, B.[1]

[1] Department of Poultry and Rabbit Diseases, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Switzerland
[2] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland

Feral pigeons, common wood pigeons and Eurasian collared doves are the most common representatives of the *Columbidae* family in Switzerland and are usually found in highly populated, urban areas. They are known carriers of various chlamydial species including *Chlamydia* (*C.*) *psittaci, C. avium, C. abortus, C. pecorum* and *C. trachomatis*. Taking into consideration that *C. trachomatis* primarily infects humans and that both *C. abortus* and *C. psittaci* are zoonotic agents, pigeons may present a human health hazard. Previous studies showed considerable differences in the prevalence of *C. psittaci* in Swiss feral pigeons (3.3% to 41.7% depending on city). In this study, 420 pigeons (331 feral pigeons, 30 domesticated pigeons, 33 Eurasian collared doves, 26 common wood pigeons) from several geographic locations in Switzerland were investigated. Samples consisted of pooled swabs (choana, cloaca; n = 184), liver samples (n = 52), and paired swab and liver samples (n = 184). DNA from all 604 samples was extracted and screened for *Chlamydiaceae* using a family-specific 23S ribosomal RNA (rRNA) real-time PCR. Positive samples (n = 91) were further analysed using a species-specific 23S rRNA Arraymate microarray assay. Samples revealing single infections with *C. psittaci* (n = 52) were not further processed.

The remaining *Chlamydiaceae*-positive samples were identified as *C. avium* positive (n = 5), *C. avium* and *C. psittaci* positive (n = 1), or could not be further classified (n = 33). For unclassified as well as all *C. avium*-positive samples, the 298-base pair amplicon of the *Chlamydiales* 16S rRNA signature gene was sequenced. Preliminary results confirmed three of the five *C. avium* samples, while analysis of the remaining *C. avium* samples is still pending. The mixed infection and seven of the unclassified samples were identified as *C. psittaci*. The remaining 26 samples yielded no clear result, but 25 were identified as *C. psittaci* according to a specific *C. psittaci* real-time PCR targeting the outer membrane protein A (*ompA*). The *Chlamydia* species could not be further classified in one sample.

In total, 15.1% (n = 91) of all samples were positive for *Chlamydiaceae.* Of these, 83 could be identified as single infections with *C. psittaci*, five as single infections with *C. avium* and one as mixed infection with *C. avium* and *C. psittaci.* One sample could not be further classified. The highest infection rates were detected in feral pigeons (65/331; 19.6%) and domesticated pigeons (5/30; 16.7%), while common wood pigeons (2/26; 7.7%) and Eurasian collared doves (1/33; 3.0%) showed lower infection rates.

The high prevalence of *C. psittaci* in domestic and feral pigeons not only elucidates the infection risk for pigeon fanciers, but shows that pigeons may present a reservoir of infection to wildlife and livestock especially during competitions.

Rapid transmission of *Chlamydia gallinacea* in a layer hen flock

Heijne, M.[1]; Goot, J. A. van der[1]; Roest, H. I. J.[1]; Koets, A.[1,2]; Velkers, F. C.[2]

[1] Department of Bacteriology and Epidemiology, Wageningen Bioveterinary Research, Lelystad, The Netherlands
[2] Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Chlamydia gallinacea is an obligate intracellular bacterium and a member of the family of *Chlamydiaceae*. *C. gallinacea* appears to be highly prevalent in commercial poultry in different countries around the world (1, 2). Its pathogenicity in poultry has not been fully proven, but a reduction in weight gain in broilers has been shown (2). Although the bacterium is genetically diverse, it is not known whether this diversity results in differences in virulence (3). Currently no epidemiological data are available on the within- and between flock transmission and duration of excretion of this bacterium.

To gain more insight in the transmission of *C. gallinacea* in chickens, we performed a study in a layer flock kept at the Faculty of Veterinary Medicine in Utrecht, The Netherlands. A flock of 18-week old layers was obtained from a commercial laying hen rearing farm and randomly distributed over two pens (A and B) with 25 hens per pen. No data were available on the presence of *C. gallinacea* during the 18 week rearing period, but serum samples were collected at day 0 (day of arrival) for *Chlamydia*

antibody testing. At day 0 per pen three *C. gallinacea* PCR positive hens of 40 weeks of age were added. The positive hens originated from a *C. gallinacea* positive layer flock at the same facility. All hens were sampled daily from day 0 until day 14 with individual cloacal swabs. The swabs were stored at –20 °C until further processing. DNA was isolated with a MagNA Pure® LC and tested using a generic *Chlamydiaceae*-PCR targeting the 23S rRNA gene (4). Samples with a Ct value up to 40 were considered positive and samples with a Ct value above 40 were considered negative.

At day 0 all 50 newly arrived hens tested negative for *Chlamydia* DNA. At day 1 one hen (4%) tested positive in pen A and three (12%) in pen B. The number of PCR positive hens further increased till day 14 when all hens in pen A (100%) and 21 (84%) in pen B tested positive for *Chlamydia* DNA. These results demonstrate that *C. gallinacea* spreads very rapidly within a *C. gallinacea* PCR negative flock. During this 14 day period no mortality or clinical signs were observed. Currently follow-up testing is in progress to evaluate the antibody status at day 0 and the duration of excretion after day 14.

References

1. Heijne M, van der Goot JA, Fijten H, van der Giessen JW, Kuijt E, Maassen CBM, et al. A cross sectional study on Dutch layer farms to investigate the prevalence and potential risk factors for different *Chlamydia* species. PloS one. 2018;13(1):e0190774.
2. Guo W, Li J, Kaltenboeck B, Gong J, Fan W, Wang C. *Chlamydia gallinacea*, not *C. psittaci*, is the endemic chlamydial species in chicken (*Gallus gallus*). Scientific reports. 2016;6:19638.
3. Guo W, Jelocnik M, Li J, Sachse K, Polkinghorne A, Pannekoek Y, et al. From genomes to genotypes: molecular epidemiological analysis of *Chlamydia gallinacea* reveals a high level of genetic diversity for this newly emerging chlamydial pathogen. BMC genomics. 2017;18(1):949.
4. Ehricht R, Slickers P, Goellner S, Hotzel H, Sachse K. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Molecular and cellular probes. 2006;20(1):60-3.

Whole-genome sequencing of avian *Chlamydia abortus* strains isolated from wild birds

Szymańska-Czerwińska, M.[1]; Zaręba, K.[1]; Mitura, A.[1]; Laroucau K.[2]; Niemczuk K.[1]

[1] Department of Cattle and Sheep Diseases/Laboratory of Serological Diagnosis, National Veterinary Research Institute, Pulawy, Poland
[2] University Paris-Est, Anses, Animal Health Laboratory, Bacterial Zoonoses Unit, Maisons-Alfort, France

For a long time, *C. psittaci* had been considered as the sole chlamydia species harbored by birds. Recent advances in molecular diagnostic resulted in discovery of new species,

also species known to infect mammals e.g. *C. abortus* have been described in birds. Recently has been proved that *C. abortus* species includes not only the classical strains isolated from mammals but also avian isolates called avian *Chlamydia abortus* (genotype G1, G2 and 1V). This study reports whole-genome sequencing of three genotypes of avian *C. abortus*.

Three avian *C. abortus* strains representing genotypes: G1, G2 and 1V were inoculated into BGM cell culture. QIAamp DNA Mini Kit (Qiagen, Germany) was used for the DNA extraction. The DNA were subjected to host methylated DNA depletion using NEB-Next Microbiome DNA Enrichment Kit (New England Biolabs, USA) prior multiple displacement amplification. Deep sequencing was carried out on an Illumina Platform (MiSeq sequencer). Read quality was assessed through FASTQC and reads were trimmed for adaptors and quality using Trimmomatic. Nonchlamydial reads pertaining to host DNA (from BGM cells) were identified through mapping to the Buffalo Green Monkey using BWA (0.7.15). The remaining reads assembled using SPSdes v. 3.11.1 with k-mer values of 21, 33, 55, 77, 99, 127. The genome characteristic was presented in the Table. The metagenomics sequence data obtained for avian *C. abortus* (G1, G2 and 1V) chromosome and plasmid was deposited in European Nucleotide Archive under accession numbers as a part of bioproject PRJEB26715. The combined chromosomal contigs for three genotypes were predicted to encode 1083, 1089 and 1080 genes respectively for G1, G2 and 1V. The %GC content in all genomes was comparable to other chlamydia genomes.

Table. Genome characteristic of avian *C. abortus*

	Avian *C. abortus*					
	Genotype G1		**Genotype G2**		**Genotype 1V**	
	chromo-some	plasmid	chromo-some	plasmid	chromo-some	plasmid
No. contigs	1	1	1	1	6	1
Length (bp)	1,141,702	7,680	1,132,456	7,683	1,151,065	7,680
GC content (%)	39.8	33.0	39.6	32.56	39.9	33.0
No. ORFs		27		27		29
Mean read coverage	~124x	~140x	~37,5x	~102,5x	~76,38x	~304,5x
No CDS on plasmid	-	8	-	8	-	8

To assess the genetic relationship between avian *C. abortus* and other chlamydia species, the scheme of Pillonel et al. (2015) was used. Based on the sequence identities of the analysed genes all analysed genotypes should be classified as *C. abortus*. It should be highlighted that percentage of sequence identity for RpoN and FtsK genes is slightly below the threshold determined by Pillonel. For PepF incomplete match was obtained. An extrachromosomal plasmid typical for most of *C. psittaci* strains was detected in these genomes. The chlamydial plasmid is normally organized with eight open reading frames (ORFs) while avian *C. abortus* have 27 or 29. The plasticity zone (PZ) region was analysed in comparison to other chlamydial species. The structure and size of PZ differs within *Chlamydiaceae* family. High level of genetic diversity is

connected with rapid evolution of putative virulence factors assembled in chlamydial PZs. The plasticity zone of G1, G2 and 1V is composed of genes required for several biochemical pathways such as Acetyl-CoA-carboxylase (accBC) and purine and pyrimidine biosynthesis genes (guaAB-add). When compared with other chlamydial species, PZs of avian *C. abortus* are most similar to the *C. abortus* but the adherence factor/cytotoxin genes are present. Their PZ are smaller than most of chlamydial species. No tryptophan operon (trpAB) was detected in the plasticity zone of avian *C. abortus* strains.

Genomic analysis of an intermediate *C. psittaci–C. abortus* strain

Longbottom, D.[1]; Wright, F.[2]; Livingstone, M.[1]; Beeckman, D.[3]; van der Ende, A.[4]; Vanrompay, D.[5,*]; Pannekoek, Y[4,*]

[1] Moredun Research Institute, Pentlands Science Park, Edinburgh, UK
[2] Biomathematics & Statistics Scotland, Invergowrie, Dundee, UK
[3] Department of Molecular Biotechnology, Faculty of Bioscience Engineering, University of Ghent, Ghent, Belgium
[4] Department of Medical Microbiology, Amsterdam University Medical Center, Amsterdam, The Netherlands
[5] Department of Animal Science and Aquatic Ecology, Faculty of Bioscience Engineering, University of Ghent, Belgium
* Joint senior authorship

Chlamydia (*C.*) *abortus* has long been recognized to be descended from classical *Chlamydia psittaci* (*C. psittaci*) strains, with a jump in the main host species from birds to mammals, in particular livestock species. Recent publications have provided evidence of the diverse nature of *C. psittaci* species, which have been shown to possess around 48,000 variable sites [1] and undergo extensive recombination, in stark contrast to *C. abortus* species where little variation has been observed (approx. 6,000 variable sites) and no or little evidence of recombination (0.9% homoplasic single nucleotide polymorphisms (SNPs)) [2]. This suggests that *C. abortus* strains are very similar genetically, with only two known variant strains from Greece (LLG/POS) that differ by around 3,000 SNPs from the main group of strains [2]. There is now emerging evidence of other strains that appear to be intermediary between *C. psittaci* and *C. abortus*, suggesting that they are recent evolutionary ancestors of *C. abortus* [3–5]. This includes strain 84/2334 that was isolated from an imported yellow-crowned Amazon parrot in Germany [6] that was initially classified as a *C. psittaci* strain and subsequently suggested to be a missing link in the divergence of *C. psittaci* into *C. abortus* [3]. Here we describe the genomic sequencing and analysis of this strain suggesting that it is a very recent ancestor of *C. abortus*. The strain additionally carries an extrachromosomal plasmid, which although inherent in most *C. psittaci* strains, has not been found in any *C. abortus* strains to date. This would make this the first known *C. abortus* strain to carry a plasmid.

References

1. Read et al. 2013. mBio 4, 604–612.
2. Seth-Smith et al. 2017. BMC Genomics 18, 344.
3. Van Loock et al. 2003. Int J Syst Evol Microbiol 53, 761–770.
4. Joseph et al. 2015. Genome Biol Evol 7, 3070–3084.
5. Szymańska-Czerwińska et al. 2017. PLoS One 12, e0174599.
6. Vanrompay et al. 1997. Res Microbiol 148, 327–333.

Population structure, phylogeny and functional genomics of *Chlamydiales*

Pannekoek, Y.; van der Ende, A.

Amsterdam UMC, University of Amsterdam, Department of Medical Microbiology, Amsterdam, The Netherlands

Multi Locus Sequence Typing (MLST), using partial sequence analysis of seven housekeeping genes, has become the number one typing approach for epidemiological and population structure analyses of microorganisms. The selected genes for MLST are not adjacent to genes under selective pressure or hypothetical genes and are separated widely on the chromosome. For each locus that is analyzed, differences in DNA sequences present within a species are assigned as distinct alleles. Next, the combination of the identified alleles at each of the loci defines the allelic profile or sequence type (ST) for each individual isolate. By using this strategy, robust data are generated that can be used to compare strains via a publicly available electronic network.

A decade ago, we have introduced a MLST typing scheme for *Chlamydiales* (Pannekoek et al., 2008). Up to date, 208 STs distributed among 11 species of *Chlamydiae* isolated from 17 different mammalian and 18 bird species are defined. These can be accessed at PubMLST (https://pubmlst.org/chlamydiales/). In addition to MLST sequences, this website also hosts the complete annotated genome sequences of 562 isolates and facilitates whole genome comparison revealed by gene-by-gene approaches[1]. Here we will provide an overview of the current state and tools available at PubMLST to study the population structure, phylogeny and functional genomics of *Chlamydiales* with focus on host-*Clamydiae* species relationship.

Reference

1. Maiden MC and Harrison OB. Population and functional genomics of *Neisseria* revealed by gene-by-gene approaches. J Clin Microbiol 2016;54(8):1949-55.

POSTER PRESENTATIONS

The Human Immunodeficiency Virus (HIV) drugs dolutegravir and elvitegravir inhibit *Chlamydia trachomatis* and *C. muridarum* development in culture

Yakoob, H.[1]; Liu, C.[2]; Grimm, M.[3]; Slade, J.[4];
Berry, A.[2]; Kintner, J.[2], Whittimore, J.[2]; Schoborg, R.[2]

[1] Virginia Commonwealth University School of Medicine, Richmond, VA, USA
[2] Department of Biomedical Sciences, Center for Infectious Disease, Inflammation and Immunity, Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA
[3] University of Tennessee College of Nursing, Knoxville, TN, USA
[4] Department of Environmental and Global Health, Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA

Introduction: As of 2015, there were ~1.1 million HIV-infected individuals in the United States and 37 million world-wide, ~5% of whom are also *C. trachomatis*-infected at any one time. HIV infection is treated with anti-retroviral therapy (ART), in which combinations of different anti-retroviral drug classes are used to suppress viral replication. The integrase strand transfer inhibitors (INSTIs) raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG), which inhibit HIV integrase enzyme activity, are widely-used ART components. EVG is structurally similar to the quinolone antibiotics, which inhibit bacterial gyrase enzymes and are considered "second-line" anti-chlamydials. Thus, we hypothesized that EVG (and perhaps other INSTIs) have anti-chlamydial activity.

Methods and Results: Human genital epithelial cell cultures were infected with *C. trachomatis* or *C. muridarum*. Physiologically-relevant concentrations [*ie.* the serum concentration of each drug obtained after a standard dose in HIV-infected patients] of RAL (1.5 μg/ml), EVG (1.7 μg/ml), or DTG (3.34 μg/ml) were then added to each culture. DTG exposure completely eliminated: i) inclusion formation; and ii) production of *C. trachomatis* and *C. muridarum* infectious EB in sub-passage assays. EVG reduced production of chlamydial EB ~85% but did not inhibit inclusion formation. As EVG did not inhibit inclusion formation frequency or gross morphology, we examined EVG-exposed chlamydial developmental forms by transmission electron microscopy. EVG exposure did not alter RB morphology, the number of different chlamydial developmental forms, or the EB/RB ratio. Thus, EVG did not prevent RB to EB conversion. Finally, RAL exposure had no observable effect on chlamydial development at any concentration tested.

Discussion: Of the INSTIs tested, DTG had the strongest anti-chlamydial activity and presumably prevented EB formation by inhibiting inclusion development. Because EVG: i) did not inhibit inclusion formation; ii) did not alter either EB number or EB/RB ratio; and iii) did significantly reduce EB infectivity in sub-passage assays, we conclude that EVG does not prevent RB to EB conversion but reduces EB infectivity. Thus, it seems likely that EVG and DTG inhibit different processes in chlamydia

development — despite our prediction that, like quinolone antibiotics, INSTIs would inhibit chlamydial gyrase. Notably, other anti-retrovirals (tenofovir and emtricitabine) reduce Herpes Simplex Virus acquisition, providing "proof of concept" that anti-retrovirals may inhibit multiple pathogens *in vivo*. If DTG similarly inhibits inclusion and infectious chlamydial EB production *in vivo*, individuals on DTG-containing ART regimens would be expected to: i) be more resistant to chlamydial infection; and/or ii) have lower risk of transmitting *C. trachomatis*. Thus, this work will facilitate future studies to: i) define the target enzyme/pathway (host or chlamydial) by which EVG and DTG alter chlamydial development; and ii) determine whether INSTI-treatment impacts chlamydia transmission or disease severity in HIV patients.

Plasmid loss in *Chlamydia muridarum*

Cousins, E.[1]; O'Neill, C.[1]; Marsh, P.[1,2]; Clarke, I. N.[1]

[1] Molecular Microbiology Group, Faculty of Medicine, University of Southampton
[2] Public Health England Laboratory, Southampton General Hospital

The phenotype of plasmid loss *in vitro* for *Chlamydia muridarum* (*C. muridarum*) has been well described and includes reduced infectivity, inability to accumulate glycogen and inclusions that present with a translucent centre resembling a "bullseye". The causative mechanism of plasmid loss is unknown as well as the impact of this phenomenon in genetic studies. Vectors for *Chlamydia trachomatis* (*C. trachomatis*) transformation commonly utilise the β-lactamase gene to facilitate selection of resistant transformants with β-lactam antibiotics such as penicillin. pGFP::Nigg, a vector developed for transformation of *C. muridarum* strain Nigg, contains Nigg plasmid coding sequences 1–8, the gene for green fluorescent protein (GFP) fused to the chloramphenicol acetyl transferase coding sequence and a separate β-lactamase gene. Preliminary investigations of *C. muridarum* strain Nigg P-transformed with pGFP::Nigg (Nigg P-/pGFP::Nigg) under penicillin selection, indicated plasmid loss where some inclusions did not express GFP. Therefore, the aim was to investigate whether plasmid maintenance is more stable under selection with chloramphenicol. Chloramphenicol has been used to obtain stable chloramphenicol resistant transformants in *C. trachomatis* but never in *C. muridarum*.

Nigg P-/pGFP::Nigg was serially passaged in McCoy cell monolayers under selection with chloramphenicol (1 µg/ml final concentration) until a consistent MOI = 1 was obtained for three passages for analysis. Wild type plasmid-bearing strain, Nigg P+, was also grown (without selection) for comparison. In Nigg P+, inclusion morphology demonstrated 11%, 8% and 14% bullseye inclusions at each passage at MOI = 1. Plasmid to chromosome copy number ratios of Nigg P+ at each passage were 0.5, 0.9 and 0.5 ($p = 0.18$) and in Nigg P-/pGFP::Nigg were 1.1, 0.7 and 0.9 ($p = 0.08$) showing stable partitioning and replication of the plasmid due to selection by chloramphenicol. GFP expression enabled transformants to be visualised under UV light. No non-green inclusions (indicating plasmid loss) were seen despite observation of the bullseye

phenotype, in contrast to published findings of *C. trachomatis* under penicillin selection. These findings demonstrate that chloramphenicol can be used to stably passage *C. muridarum* transformants with a minimal amount of plasmid loss. Future work aims to determine whether stable plasmid maintenance is achievable with penicillin selection in *C. muridarum*. Chloramphenicol selection in *C. muridarum* may improve transformation efficiency through improved plasmid maintenance.

Clinical outcome of ocular infection with *Chlamydia suis* S45 in nursery piglets

Unterweger, C.[1]; Setudeh, S.[1]; Inic-Kanada, A.[2];
Stein, E[2]; Barisani-Asenbauer, T.[2]; Ladinig, A.[1]

[1] University Clinics for Swine, Vetmeduni, Vienna, Austria
[2] OCUVAC — Institute of Specific Prophylaxis and Tropical Medicine,
Center for Pathophysiology, Infectiology and Immunology;
Medical University of Vienna; Vienna, Austria

Purpose. *Chlamydia (C.) suis* infection has been associated with several clinical signs in pigs including rhinitis, pneumonia, enteritis, reproductive disorders and inferior semen quality as well as conjunctivitis. In the field, intensively kept pigs seem to be particularly predisposed to ocular infection, although they are often unnoticed due to the unsatisfying diagnostic methods available. Zoonotic transmission from pigs to employees of a slaughterhouse have been confirmed in the past. In contrast to humans, little is known about the pathogenesis of *Chlamydia* associated conjunctivitis in pigs.

Materials and Methods. After preparation of the inoculum (10^9 inclusion forming units/ml), *C. suis* strain S45 was instilled into the right lower conjunctival sac (100 µl) of five 6 week-old piglets free of PRRSV and other immunosuppressing pathogens. Four piglets served as non-infected controls. Starting seven days prior to infection, a daily check of the eyes and overall health including faecal scoring was performed until necropsy 21 days after infection (SD21). Weights were recorded once a week.

Results. None of the infected or control piglets developed severe clinical signs at any time during the study. Between SD 14 and 17, infected piglets showed pasty to liquid faeces, while faeces of control piglets had a normal consistency. Left and right eye lids of infected piglets showed moderate to severe reddening for 3 days after infection. Upper and lower eyelids were oedematous between SD 2 and 7 as well as from SD 12 until termination (SD 21). Reddening of the tarsal conjunctivae could only be seen on SD 3 and SD 7. Serous discharge was recorded at three timepoints: between SD 1 and 3 (low degree), on SD 10 (high degree) and between SD 14 and 21 (high degree). No differences in average daily weight gain was seen between infected and control pigs.

Conclusion. Single ocular infection of healthy piglets with *C. suis* at high dose affects mainly the ocular surface local health. The most remarkable signs were the biphasic eyelid edema and discontinuous serous discharge. Nevertheless, diarrhea was also seen

in infected piglets. Further investigations are needed to examine the role of infection load and repeated infection on development of further pathologies.

Effect of sex hormones on the establishment of the immune response induced by vaccination against *Chlamydia abortus* in a mouse model

A. Murcia-Belmonte[1]; D. Álvarez[1]; N. Ortega[1]; J. A. Navarro[2]; E. Gómez-Lucía[3]; A. J. Buendía[2]; L. Del Rio[1]; J. Salinas[1]; M. R. Caro[1]

[1] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, España
[2] Departamento de Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Murcia, España
[3] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, España

Chlamydia abortus produces ovine enzootic abortion (OEA). One especially intriguing aspect of the pathology of this disease is that infection of non-pregnant sheep leads to a state of latency. Clinical signs are not observed until the ewes become pregnant. In that moment, the organism colonizes the placenta and multiplies eventually causing abortion in the last weeks of gestation. After abortion, animals acquire protective immunity, although they become carriers and will shed the organism in the following oestruses. This process of latency/active multiplication that leads to the recrudescence of *C. abortus* at certain moments of pregnancy and oestrus cycle, suggests that sex hormones might play an important role on the immune system that could affect the success of a chlamydial clearance and also jeopardizing the effectiveness of a vaccination. Previous studies have shown the influence of sex hormones in *C. abortus* infections but not in the development of immune response elicited during vaccination. Therefore, the goal of this study is to know the effect of 17β-estradiol and progesterone in the immunisation process conferred by an experimental vaccine against *C. abortus* previously tested in mouse and ovine experimental models and by a commercial vaccine.

To achieve this objective, 45 ovariectomised Swiss OF1 mice were randomly allocated in 3 groups: vaccinated with an experimental vaccine (EV) vaccinated with a commercial vaccine (CV) and non-vaccinated (NV). Vaccinated mice group was immunized 43 and 29 days before infection with the EV or with the CV used in this study. All mice were subcutaneously administered either 3 mg of progesterone, 3 μg of 17β-oestradiol, or only sesame oil (control group) 1 day before the first vaccination and 1 and 7 days after it. In addition, they were also treated 1 day before boosted and 1 day after it. Therefore 29 days after boosting, animals were challenged intraperitoneally with 5 x 10^6 inclusion-forming units of *C. abortus* strain AB7 and they were sacrificed at day 4 post-infection. The morbidity was measured as weight loss and samples of sera were collected for antibodies analyses. In addition, liver were

collected the day of sacrifice for chlamydial quantification and histopathological and immunohistochemical analyses.

The preliminary results showed that chlamydial load was lower in EV mice, confirming the effectiveness of the experimental vaccine employed. On the other hand, chlamydial load was higher in progesterone-treated animals than in oestradiol-treated mice in vaccinated and non vaccinated groups. This could suggest that in mice the progesterone in high doses could interfere on the establishment of the immune response induced by vaccination against *C. abortus* whereas oestradiol could enhance it. However, further experiments are necessaries to get more robust conclusions.

Efficacy of a new inactivated vaccine against *Chlamydia abortus* and *Salmonella enterica* serovar Abortusovis experimental challenges of pregnant ewes

Fontseca, M.[1]; Montbrau, C.[1]; Salinas, J.[2]; Caro, M. R.[2];
Gutierrez-Exposito, D.[3]; Arteche, N.[3]; Perez, V.[3]; Benavides, J.[3];
Bezos, J.[4,5]; García-Seco, T.[5]; Domínguez L.[4,5]; Sitjà, M.[1]; March, R.[1]

[1] HIPRA Scientific S.L.U., Amer, Spain
[2] Animal Health Department, Faculty of Veterinary, University of Murcia, Spain
[3] Instituto de Ganadería de Montaña (CSIC-Universidad de León), León, Spain
[4] Department of Animal Health, Faculty of Veterinary Medicine,
Complutense University of Madrid, Spain
[5] VISAVET Health Surveillance Centre, Complutense University of Madrid, Spain

The aim of the present study was to evaluate the efficacy of a new inactivated vaccine (HIPRA, Spain) against *Chlamydia abortus* and *Salmonella enterica* serovar Abortusovis (SAO) after an experimental subcutaneous challenge of those pathogens with heterologous strains in pregnant ewes.

Eighty seven no-pregnant ewes were randomly assigned to one of the two groups: Group 1 (n = 36), vaccinated with the inactivated vaccine; Group 2 (n = 51), mock-vaccinated with phosphate buffered saline (PBS) as a control group. Treatment administration of both groups was done intramuscularly 5 weeks and 2 weeks before mating. Ten ewes from group 2 (control) were used as a sentinel group (not infected). All these ewes were confirmed as pregnant and were distributed in two experimental challenges (except sentinel group) to assess the efficacy against *C. abortus* and SAO. Nineteen vaccinated and twenty-one control animals were experimentally infected by subcutaneous injection with a virulent heterologous strain of *C. abortus*. This challenge was carried out at approximately 75 days after mating. The third group of 10 pregnant ewes was monitored as a sentinel group. The others 17 vaccinated and 20 control animals were experimentally infected by subcutaneous injection with a virulent heterologous strain of SAO. The challenge was carried out at approximately 90 days after mating. Reproductive disorders (i.e. abortions, stillbirths or neonatal deaths), shedding and antibody response were registered and monitored after both experimental

challenges. In *C. abortus* challenge, shedding was monitored just before challenge, at abortion/parturition day and on the following three weeks after parturition or abortion. On the other hand, shedding of animals infected with SAO was monitored just before challenge, from 10 to 40 days post-challenge and at abortion/parturition day.

After *C. abortus* experimental challenge, vaccinated animals showed a statistically significant reduction ($P < 0.05$) of reproductive disorders caused by *C. abortus* compared to control animals (11% vs 43%, respectively). The shedding of vaccinated animals had a significant reduction ($P < 0.05$) compared to control group in terms of percentage of positive samples at parturition/abortion day (39% vs 86%, respectively) and from parturition/abortion to three weeks after (19% vs 37%, respectively). The amount of shedding ($DICT_{50}$/ml) was also significantly ($P < 0.05$) lower in vaccinated group compared to control group at parturition or abortion day (2.0 ± 0.62 vs 5.1 ± 0.51 log $DICT_{50}$/m, respectively) and during the following three weeks (2.5 ± 0.54 vs 5.1 ± 0.31 log $DICT_{50}$/ml, respectively). Vaccinated ewes had significant ($P < 0.05$) greater antibodies titers than control animals. After challenge, both groups had similar levels of antibodies. Referring SAO experimental challenge, vaccinated animals had a statistically significant reduction ($P < 0.05$) of reproductive disorders compared to control animals (29% vs 65%, respectively). Referring to shedding, vaccinated animals showed a significant reduction ($P < 0.05$) in the percentage of positive samples at abortion/parturition day (29% vs 70%, respectively) and from 10 to 40 days after challenge (11% vs 19%, respectively). Furthermore, the amount of shedding (CFUs/ml) was significantly ($P < 0.05$) lower in vaccinated animals compared to control animals at abortion/parturition day (1.8 ± 0.71 vs 4.56 ± 0.70 log CFUs/ml, respectively) and from 10 to 40 days after challenge (3.0 ± 0.84 vs 5.7 ± 0.67 log CFUs/ml, respectively). Vaccinated ewes had significantly ($P < 0.05$) higher antibodies titers compared to control animals before the challenge. After challenge, both groups had similar levels of antibodies.

Overall, the results obtained allow to conclude that vaccination with the new inactivated vaccine, according to the recommended vaccination schedule, significantly reduces reproductive disorders and shedding after the experimental challenge with a virulent strain of *C. abortus* or SAO.

Protective efficacy of a new inactivated vaccine against *Chlamydia abortus* in a pregnant mouse model

Montbrau, C.[1]; Fontseca, M.[1]; Roca, M.[1]; Ortega, N.[2]; Del Río, L.[2]; Alvarez. D.[2]; Murcia-Belmonte, A.[2]; Caro, M. R.[2]; Salinas, J.[2]; March, R.[1]; Sitjà, M.[1]

[1] HIPRA Scientific S.L.U., Amer, Spain
[2] Animal Health Department, Faculty of Veterinary, University of Murcia, Spain

Chlamydia abortus (*C. abortus*) is the etiological agent of ovine enzootic abortion (OEA), an economically important disease in many countries. This pathogen is a gram-

negative obligate intracellular bacterium able to colonize the placenta producing inflammation, which may induce abortion in the last third of pregnancy. Furthermore, its zoonotic risk has been reported in pregnant farmers or abattoir workers. Mouse models have been widely used to study both the pathology of the disease and the role of immune cells in controlling infection. Moreover, this animal experimental model has been considered a useful tool to evaluate new vaccine candidates and adjuvants that could reduce abortion and fetal death. This study evaluates the grade of protection of one new inactivated vaccine against experimental infection of *C. abortus* in pregnant mouse model.

Seventy female OF1 mice were randomly distributed in four groups. Group 1 (n = 20) was immunized subcutaneously with 0.2 ml (two doses two weeks apart) of new inactivated vaccine of *C. abortus* (A22 strain) and *Salmonella enterica* serovar Abortusovis (Hipra). Group 2 (n = 20) was immunized subcutaneously with 0.2 ml of an experimental inactivated vaccine (UMU) with the AB7 strain of *C. abortus* designed by the research group of University of Murcia, that has previously shown an adequate protection, used as protective efficacy control. Group 3 (n = 20) received PBS using the same immunization protocol as group 1 and 2. Group 4 (n = 10) was a sentinel group. On day 34–37 all mice were mating. Then, on day 47, animals of group 1, 2 and 3 were infected intraperitoneally (5×10^4 IFUs) with *C. abortus* strain AB7 (heterologous to group 1 and homologous for group 2) isolated initially from an ovine abortion in France.

Serum samples were obtained on days 0 (1st vaccination), 14 (2nd vaccination), 47 (challenge day), 51 and 58. A minimum of five animals were analyzed per group except in the third extraction (day 47) where only three-four animals were used per group. To evaluate the humoral response to *C. abortus*, the antigen produced in yolk sacs of embryonated eggs was used in order to avoid non-specific reactions against the proteins of the McCoy cells, which was used to prepare the vaccines. The serological results obtained demonstrate that vaccinated animals with group 1 significantly greater ($P < 0.05$) titre of sera antibodies before challenge compared to mock-vaccinated group. After infection both groups (1 and 2) significantly greater ($P < 0.05$) compared to mock-vaccinated.

From challenge until parturition reproductive disorders were monitored and the course of infection was also evaluated from liver and uterus samples obtained from dead animals, detecting and counting (IFUs) the presence of *C. abortus*.

Vaccinated animals from group 1 and 2 showed a statistically significant reduction ($P < 0.05$) of reproductive disorders caused by *C. abortus* compared to control animals (25% and 0% vs 100%, respectively). Even the number of pups was significantly ($P < 0.05$) greater in the vaccinated groups (group 1 = 8.6 and group 2 = 12.9) compared to group 3 (1.6 pups). No differences were observed among vaccinated and sentinel groups. In terms of detection and quantification (IFUs) of *C. abortus*, vaccinated groups described a significant reduction ($P < 0.05$) compared to control infected group.

Overall, the results obtained allow to conclude that vaccination with the new Hipra inactivated vaccine, significantly reduces reproductive disorders and shedding of *C. abortus* caused by an experimental infection with a heterologous virulent strain of *C. abortus* in mice, and the new vaccine could be a good candidate to be assayed in the natural host, the sheep.

Genomic analysis of *pmps* and *plasticity zone* of two *C. pecorum* isolates from a chamois and a water buffalo

Rigamonti, S.[1]; Floriano, A. M.[2]; Longbottom, D.[3]; Scaltriti, E.[4]; Comandatore, F.[2]; Casadei, G.[4]; Capucci, L.[5]; Donati, M.[6]; Vicari, N.[1]; Magnino, S.[1]

[1] National Reference Laboratory for Animal Chlamydioses, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Pavia, Italy
[2] Centro per la Ricerca Pediatrica "Romeo ed Enrica Invernizzi", Dipartimento di Scienze Biomediche e Cliniche L. Sacco, University of Milan, Milan, Italy
[3] Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh, Scotland, United Kingdom
[4] IZSLER, Department of Parma, Parma, Italy
[5] IZSLER, Unit of Proteomics, Brescia, Italy
[6] DIMES, Microbiology, University of Bologna, Bologna, Italy

Two *C. pecorum* isolates stored at the Italian NRL for Animal Chlamydioses, PV7855 from the lung of a chamois affected with pneumonia and PV6959 from the brain of a water buffalo calf with encephalomyelitis, were grown respectively in LLC-MK2 and McCoy cells, their elementary bodies were purified according to the sucrose gradient separation protocol and their whole genome sequences were obtained with MiSeq System (Illumina). Analyses focused on two polymorphic regions of chlamydiae, the gene cluster of polymorphic membrane proteins (*pmps*) and the plasticity zone (PZ).

We compared the two isolates against the reference strains E58 and PV3056/3 and against each other with the Artemis Comparison Tool (ACT). The E58 strain was isolated from the brain of a calf with encephalomyelitis in the US and PV3056/3 was isolated from a dairy cow with endometritis in Italy. The genomes of PV3056/3, PV6959 and PV7855 strains were aligned to the reference genome E58 using Mauve. Then single nucleotide polymorphisms (SNPs) were called and annotated (at the nucleotide and amino acid level) using an in-house Perl script. Comparisons of coding sequences between genomes were made using the alignment tool ClustalW.

PV7855 showed a much higher homology with E58 (192 SNPs in total, of which 125 are non-synonymous substitutions) than with PV3056/3 (15,198 SNPs in total, of which 6,639 are non-synonymous). In particular, the amino acid alignment of all *pmps* showed a homology between 99 and 100% when compared to E58, and a homology between 85.49 and 96% when compared to PV3056/3. As to the PZ of PV7855, we focused our analyses on the phospholipase genes (*pld1*, *pld2*, *pld3* and *pld4*) and the

toxin B genes (1 and 2). Also in this case PV7855 is more similar to E58 than to PV3056/3: indeed *pld1*, *pld2*, *pld3* and *pld4* show a homology of 100% in the amino acid sequences, *pld1* being the most divergent compared to PV3056/3, with a homology of 56.81%. Also the toxin B genes of PV7855 are more similar to the ones in E58 (99% both) than in PV3056/3 (87 and 86%, respectively).

The comparison between PV6959 and E58 showed a difference of 23,943 SNPs with 8,631 non-synonymous substitutions, while the comparison with PV3056/3 resulted in 25,799 SNPs with 9,425 non-synonymous SNPs. There are no significant differences in *pmps* region, the homology being between 78 and 98% when PV6959 is compared to E58 and between 77 and 98% when compared to PV3056/3. As to the PZ, the amino acid sequence of *pld1* has a high degree of similarity (95.93%) with the one in E58, while *pld2* and *pld3* have a much lower similarity (54.45 and 59.15%). The two toxin B genes are highly homologous (between 83 and 94%) to both reference strains. In the PZ of PV6959 we also detected a hypothetical protein which seems to be a unique gene.

The comparison between our two isolates showed that they differ by 23,985 SNPs, of which 8,664 are non-synonymous substitutions. Overall, the region of *pmps* is highly homologous and *pmp6* is the least similar *pmp* with 78.22% similarity in the amino acid sequence. The analysis of PZ is under way.

These preliminary results show that the PV7855 and PV6959 isolates have few differences in some highly variable regions of their genomes. In addition, the genomes of PV6959 and E58 (which were both isolated from animals affected with encephalomyelitis) are slightly different both as a whole and in the variable regions studied. These data suggest that the genomic regions considered in this study may not be related to tissue tropism. The investigation of further regions is ongoing.

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Presence of *Waddlia chondrophila* in drinking water systems in the Netherlands

van Dooremalen, W.[1,2]; Morré, S. A.[1,2,3]; van der Wielen, P.[4]; Ammerdorffer, A.[1,2]

[1] TubaScan Ltd, de Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands
[2] Amsterdam UMC, Vrije Universiteit Amsterdam, Department of Medical Microbiology and Infection Control, Laboratory of Immunogenetics, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands
[3] Institute for Public Health Genomics, Department of Genetics and Cell Biology, Research Institute GROW, Faculty of Health, Medicine & Life Sciences, University of Maastricht, Maastricht, The Netherlands
[4] KWR Watercycle Research Institute, Nieuwegein, The Netherlands

Introduction. *Waddlia chondrophila* is an emerging pathogen belonging to the order of Chlamydiales. This obligate intracellular bacterium was initially isolated from an aborted bovine fetus [1], and is associated with adverse pregnancy outcomes in animals

and humans [2–4]. Unlike the well-known *Chlamydia trachomatis,* that mainly spreads through sexual contact, the transmission routes of *W. chondrophila* have not yet been fully elucidated. Potential routes of infection include the consumption of milk and uncooked meat, contact with animals and possibly sexual contact [5]. Its ability to reside within a range of free-living amoebae furthermore implies a possible widespread environmental presence of *W. chondrophila* [6, 7]. Amoebae have been reported to be able to break through the water treatment barrier and enter distribution systems, where they can colonize and regrow [8]. Indeed, *W. chondrophila* DNA has been identified in drinking water sources in various European countries [9–11]. Potential *W. chondrophila* hosts are also present in Dutch drinking water [12]. Therefore, this study investigates the presence of *W. chondrophila* DNA in drinking water systems in the Netherlands.

Methods. Water samples were obtained from eleven drinking water systems throughout the Netherlands. Samples were taken at three distances from the pumping station, during both summer and winter. The water tap was flushed for 30 seconds before sampling, to make sure that microorganisms present in the local environment were flushed out. DNA was isolated and the presence of *W. chondrophila* specific DNA was determined using quantitative PCR.

Results. Two out of the eleven tested drinking water systems contained low levels (Ct > 35) of *W. chondrophila* DNA. Both pumping stations were located in coastal regions and use surface water to produce drinking water; no *W. chondrophila* DNA was found in drinking water samples derived from ground water. Samples taken in winter revealed higher levels of *W. chondrophila* DNA than those taken in summer. Furthermore, higher levels of *W. chondrophila* DNA were found in samples taken at shorter distances (7 km versus 50 km) from the pumping stations.

Conclusions Our results indicate the presence of low levels of *W. chondrophila* in Dutch drinking water systems. This is in accordance with the DNA levels found in the previous studies [9, 10]. Further research should elucidate whether this is live *W. chondrophila* that is still able to infect a host, and thus whether drinking water could be an actual transmission route for *W. chondrophila*.

References

1. Dilbeck, P. M., et al., *Isolation of a previously undescribed rickettsia from an aborted bovine fetus.* J Clin Microbiol, 1990. **28**(4): p. 814-6.
2. Baud, D., et al., *Waddlia chondrophila, a potential agent of human fetal death.* Emerg Infect Dis, 2007. **13**(8): p. 1239-43.
3. Verweij, S. P., et al., *Waddlia chondrophila and Chlamydia trachomatis antibodies in screening infertile women for tubal pathology.* Microbes Infect, 2015. **17**(11-12): p. 745-8.
4. Dilbeck-Robertson, P., et al., *Results of a new serologic test suggest an association of Waddlia chondrophila with bovine abortion.* J Vet Diagn Invest, 2003. **15**(6): p. 568-9.

5. Ammerdorffer, A., et al., *Chlamydia trachomatis and chlamydia-like bacteria: new enemies of human pregnancies.* Curr Opin Infect Dis, 2017. **30**(3): p. 289-296.
6. Michel, R., et al., *Free-living amoebae serve as a host for the Chlamydia-like bacterium Simkania negevensis.* Acta Protozoologica, 2005. **44**(2): p. 113-121.
7. Michel, R., et al., *Free-living amoebae may serve as hosts for the Chlamydia-like bacterium Waddlia chondrophila isolated from an aborted bovine foetus.* Acta Protozoologica, 2004. **43**(1): p. 37-42.
8. Thomas, J. M. and N. J. Ashbolt, *Do Free-Living Amoebae in Treated Drinking Water Systems Present an Emerging Health Risk?* Environmental Science & Technology, 2011. **45**(3): p. 860-869.
9. Codony, F., et al., *Well water as a possible source of Waddlia chondrophila infections.* Microbes Environ, 2012. **27**(4): p. 529-32.
10. Agusti, G., et al., *Presence of Waddlia chondrophila in hot water systems from non-domestic buildings in France.* J Water Health, 2018. **16**(1): p. 44-48.
11. Lienard, J., et al., *Prevalence and diversity of Chlamydiales and other amoeba-resisting bacteria in domestic drinking water systems.* New Microbes New Infect, 2017. **15**: p. 107-116.
12. van der Wielen, P. W. and D. van der Kooij, *Nontuberculous mycobacteria, fungi, and opportunistic pathogens in unchlorinated drinking water in The Netherlands.* Appl Environ Microbiol, 2013. **79**(3): p. 825-34.

Why are there different clinical outcomes in multifetal sheep infected with *Chlamydia abortus*?

Caspe, S. G[1,2,3]; Longbottom, D.[2]; Milne, E.[3]; Livingstone, M.[2]; Wattegedera, S[2]; Aitchison, K[2]; Entrican, G[2]; Sargison N.[3]; Chianini, F.[2]

[1] INTA Mercedes, CC 38, Mercedes, Corrientes, Argentina
[2] Moredun Research Institute, Pentlands Science Park, Midlothian EH26 0PZ
[3] The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG

Chlamydial abortion or Ovine Enzootic Abortion (OEA) is a zoonotic disease caused by infection with *Chlamydia abortus* (*C. abortus*). OEA is one of the most commonly diagnosed causes of infectious abortion in sheep flocks in many countries throughout Europe. The economic impact of OEA in the UK is estimated at £20 million per year [1]. This disease can have various clinical outcomes such as birth of one or more dead fetuses, weakly lambs that cannot survive and/or apparently healthy "normal" lambs, or a combination of these outcomes in cases where ewes have multifetal pregnancies [2, 3]. These different outcomes are related to the presence of placental lesions and the degree of pathological damage that has occurred due to chlamydial infection. Therefore, the objective of this study is to investigate the causes and to understand why there can be different outcomes of chlamydial infection in ewes with multifetal pregnancies.

Scotch Mule twin-bearing ewes were experimentally-infected with 2×10^6 IFU *C. abortus* and their clinical outcome recorded in terms of the viability of every lamb/fetus. Clinical outcome were classified as Dead/Dead when both foetuses were aborted (n = 3), Dead/Live when one fetus was aborted and the other one was alive (n = 3), and Live/Live when both lambs born alive (n = 2). Each ewe and its lamb(s)/fetus(es) were euthanised, necropsied, and a range of tissues (liver, lungs, brain, mammary gland and preescapular, mediastinal and lumboaortic lymph nodes), colostrum and fetal stomach contents aseptically collected for microbiological (mZN of placental smears), molecular (real-time qPCR of vaginal swabs and placental material), immunological (*in situ* hybridization and IHC on fixed and frozen tissue) and pathological analyses (IHC and histology). Uninfected twin-bearing ewes (n = 2) and lambs were also necropsied and sampled as above and served as controls. Placentas were collected from every ewe at the time of lambing/abortion, macroscopically assessed for pathognomonic OEA lesions, photographed and sampled. All the animals were bled throughout the trial and at parturition to determine the antibody response. Preliminary results of mZN, real-time qPCR and histopathology will be presented and discussed. The information generated will be used to evaluate any difference in terms of cellular infiltration, severity of lesions, and bacterial load between different animals, in order to understand the differences in clinical manifestation that can occur following *C. abortus* infection.

References

1. Longbottom, D. and L. J. Coulter, *Animal chlamydioses and zoonotic implications.* J Comp Pathol, 2003. **128**(4): p. 217-44.
2. Gutierrez, J., et al., *Monitoring clinical outcomes, pathological changes and shedding of Chlamydophila abortus following experimental challenge of periparturient ewes utilizing the natural route of infection.* Vet Microbiol, 2011. **147**(1-2): p. 119-26.
3. Livingstone, M., et al., *Pathogenic outcome following experimental infection of sheep with Chlamydia abortus variant strains LLG and POS.* PLoS One, 2017. **12**(5): p. e0177653.

Presence of *Chlamydiaceae* in different birds kept in zoos and other collections in Croatia

Horvatek Tomic, D.[1]; Quilicot, A. M.[2]; Gottstein, Z.[1];
Lukac, M.[1]; Prukner-Radovcic, E.[1]

[1] Faculty of Veterinary Medicine, University of Zagreb,
Heinzelova 55, 10000 Zagreb, Croatia
[2] College of Veterinary Medicine, Visayas State University,
ViSCA, Baybay City, Leyte, Philippines

Numerous bird species are often kept in captivity, individually or in collections of different sizes. In some occasions, although it is not recommended due to the potential disease spread, birds originating from different part of the world are kept in the close proximity to each other. If such collections are open for the public, it is necessary that the birds are tested for the presence of potential zoonotic agents.

In the Republic of Croatia, it is obligatory, according to the law, that all birds kept in zoos or other publicly available collections must be examined for the presence of *C. psittaci* at least once per year.

From 2013 to 2017, altogether 143 samples were collected from three zoos and one fauna park in Croatia and were tested for the presence of *Chlamydiaceae* and *C. psittaci*. Out of them, 12 were so called triple or cloacal swabs and the remaining were combined fecal samples. Fecal samples originated from a single bird or from up to 50 birds, kept as a single flock. The birds belonged to the orders *Accipitriformes, Anseriformes, Columbiformes, Ciconiiformes, Galliformes, Gruiformes, Musophagidae, Passeriformes, Piciformes, Psittaciformes and Strigiformes*. The samples were examined in the Laboratory for Chlamydia (CHLAMlab), at the Department of Poultry Diseases, Faculty of Veterinary Medicine University of Zagreb. The DNA was extracted by using Gene Elute Mammalian kit (Sigma, USA) and examined by reealtime PCR for *Chlamydiaceae* 23S rRNA gene, *C. psittaci* incA gene, and *C. gallinacea* enoA gene.

Out of 143 examined samples, 34 were found positive for *Chlamydiaceae* (23.77%). The majority of positive birds belonged to the orders *Psittaciformes* (15) and *Galliformes* (7). None of the samples were found positive for *C. psittaci*. One sample originated from the gallinaceous bird were found positive for *C. gallinacea*.

The presence of 23.77% *Chlamydiaceae* positive samples confirm the need for further investigation and identification of new *Chlamydiaceae* species with possible zoonotic potential among captive birds in close contact with humans.

Chlamydia abortus and *Chlamydia pecorum* infections in ruminants in Ukraine

Iuliia Krivoshei[1]; Theresa Pesch[2]; Barbara Prähauser[2];
Andreas Pospischil[2]; Vitaliy Bolotin[1]; Nicole Borel[2]

[1] National Scientific Center Institute of Experimental
and Clinical Veterinary Medicine, Kharkiv, Ukraine
[2] Institute of Veterinary Pathology, Vetsuisse Faculty,
University of Zurich, Zurich, Switzerland

Chlamydial infections in ruminants are disseminated worldwide and can cause significant economic losses. To date, chlamydial infections of ruminants caused by *C. abortus* and *C. pecorum* are subjected to particular scrutiny. *Chlamydia abortus* causes enzootic abortion in sheep and goats (EAE, or ovine enzootic abortion (OEA)). *Chlamydia pecorum* is often carried asymptomatically in the intestinal tract, but can also cause pneumonia, conjunctivitis, polyarthritis, intestinal infections, mastitis and metritis. Our objective was to study the occurrence of infections caused by *C. abortus* and *C. pecorum* in herds of domestic ruminants in different regions of Ukraine.

Vaginal (cattle, n = 5; ewes, n = 19) and conjunctival (cattle, n = 10) swabs (COPAN, Italy), intestinal organs from aborted fetuses (cattle, n = 3), milk (cattle, n = 5) and serum samples (cattle, n = 69; ewes, n = 338; goats, n = 34) were collected from farms, located in 6 different regions of Ukraine, between June 2017 and July 2018, and were tested using serological and/or molecular methods. Samples were taken from farm animals, which were showing symptoms such as late-term abortions, births of non-viable calves, metritis, arthritis and conjunctivitis and from non-symptomatic animals.

We used the ID Screen® Chlamydophila abortus Indirect Multi-species ELISA (IDvet, France) and the CHEKIT®-IDEXX Chlamydiosis Total Ab Test (IDEXX Laboratories) to detect the presence of antibodies against *C. abortus* in serum samples. The DNA was extracted using the innuPREP MP Basic Kit (Analytik Jena, Jena, Germany) according to manufacturer's instructions and was examined using quantitative real-time PCR assay (qPCR) specific for the family *Chlamydiaceae* targeting the 23S rRNA gene (Ehricht et al., 2006). The identification of chlamydial species was conducted with the 23S ArrayMate microarray assay (Borel et al., 2008).

In our study, we obtained the following results: two out of 69 serum samples from cattle were positive by CHEKIT ELISA (IDEXX Laboratories) but none were positive by ID Screen® ELISA (IDvet, France). In goats, two out of 34 serum samples were positive by CHEKIT ELISA (IDEXX Laboratories) but none by ID Screen® (IDvet, France). Testing of sheep serum sampled revealed 14 positive samples by CHEKIT ELISA (IDEXX Laboratories), nine samples were only positive by ID Screen® ELISA (IDvet, France), and 10 samples gave positive results by both tests. DNA samples were negative for *Chlamydiaceae* except two conjunctival swabs from calves. The microarray assay analysis showed that these two samples contained DNA of *C. pecorum*.

Obtained results indicate that chlamydial infections in ruminants in Ukraine are present. Constant monitoring is needed to provide us with the up-to-date data and new strategies to ensure the control of chlamydial infections in Ukraine. This work was supported by SNF within SCOPES Project (IZ74Z0_160468/1).

Serological screening for *Yersinia enterocolitica* serotypes O:3, O:6.30 and O:9 in ruminants and pigs during 2015–2017

Ganna Orekhova; Andriy Zavgorodniy; Vitaliy Bolotin

National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine

The goal: Conducting of serological screening for *Yersinia enterocolitica* serotypes O:3, O:6.30 and O:9 in ruminants and pigs during 2015-2017.

Methods: Serological screening for prevalence of *Y. enterocolitica* serotypes O:3, O:6.30 and O:9 were conducted among ruminants and pigs during 2015–2017 in Ukraine. Serum samples were obtained as part of scheduled examinations for leucosis, brucellosis and other infectious diseases on selected farms. Remaining, available sera were used for screening of intestinal yersiniosis. We studied 1,736 blood serum samples from cattle, 249 from sheep and 359 from pigs in 11 regions of Ukraine. Total number of samples was 2,344. Serum samples were tested using a Serum tube Agglutination Test (SAT). SAT were performed using three antigens from *Y. enterocolitica* serotypes O:3, O:6.30 and O:9 (TC U 46.15.091-95). Sera were diluted from 1:50 to 1:800. There was used 0.85% physiological saline solution to dilute the serum. A diagnostic SAT titer was a positive reaction scored as a 2 (++), 3 (+++) or 4 (++++) pluses at a serum dilution of 1:200 or above. Samples with reactions of (++) with serum dilutions of 1:100 titers, were considered as "weak" results.

Results: Seroprevalence for *Y. enterocolitica* of serotype O:3 among cattle was 22%, "weak" results were found in 7% of cattle. Diagnostic titers were detected in 27% of sheep and 30% was "weak" animals. Among pigs were detected 36% of positive animals and 12% of animals had reaction with the result showing ++ with serum dilution from 1:100 titers.

Serological screening for yersiniosis infection caused by the causative agent of *Y. enterocolitica* serotype O:6.30 showed 37% positive cattle and 21% "weak" animals. Positive results in sheep — 29%, "weak" result — 50%. Diagnostic titers were observed in 10% of pig and 23% showed "weak" result.

SAT with antigen of the serotype O:9 showed 56% positive cattle and 19% had "weak" result. Diagnostic titers were observed in 8% of sheep, 39% showed "weak" results. During examination of blood samples of pigs there was 39 % of seropositive animals and 11% of animals with the titers below than diagnostical.

Conclusions: High level of *Y. enterocolitica* seroprevalence was demonstrated among farm animals of industrial herds of cattle, sheep and pigs. The "weak" reactions might be connected to the circulation of low-virulence *Yersinia* isolates, which in case of reversion may pose a threat for animal and people health. The obtained results of our research showed *Y. enterocolitica* infected domestic animals that might cause contamination of animal products. It is necessary to continue surveillance regarding intestinal yersiniosis in agricultural farms in Ukraine to minimize the risk of human infection.

MLVA analysis of *Francisella tularensis* samples collected on the territory of Ukraine from 1997 to 2016

Zlenko O. B.[1]; Durr A.[2]; Schwarz J.[2]; Vydaiko N. B.[3]; Gerilovych A. P.[1]

[1] National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine
[2] Bundeswehr Institute of Microbiology, Department for Bacteriology and Toxicology, Munich, Germany
[3] Central Laboratory of Hazardous Diseases of the State Institution Ukrainian Center for Diseases Control and Monitoring of the Ministry of Health of Ukraine, Kyiv, Ukraine

Introduction. Tularemia agent — *Francisella tularensis* — is a gram negative, facultative, bacterial pathogen that can infect a large number of different mammalian species, including humans. Tularemia is an endemic disease in most European countries. Natural foci of tularemia may exist for centuries, showing themselves as periodic disease outbreaks. The epidemiological and epizootiological situation in Ukraine varied over the years and depended on the level of diagnosis and quality of the implementation of appropriate measures. The problem of tularemia infection is particularly acute in the east of the country, since large areas are currently uncontrolled.

MLVA (Multi-locus variable number tandem repeat)-analysis of the tularemia pathogens can determine its genotypic characteristics, as well as the frequency and distribution of individual genotypes on the territory of Ukraine.

Materials and methods. The 20 *F. tularensis* thermolysates for MLVA-genotyping were kindly provided by the Central Laboratory of Hazardous Diseases of the State Institution Ukrainian Center for Diseases Control and Monitoring of the Ministry of Health of Ukraine (SI UCDCM MoH of Ukraine). All samples were collected in the years 1997–2016 on the territory of Ukraine.

The genotyping and processing of data was carried out on the basis of the Institute of Microbiology of the Bundeswehr (Munich, Germany) within the framework of the Ukrainian-German Biosafety Program, using the MLVA 12 + 1 method (Vogler et al., 2009; Svensson et al., 2009). Genotyping was performed using the Genetic Analyzer 3130 (Applied Biosystems, USA) device. Data were statistically processed in

Genemapper (Thermoficher scientific, USA) and Bionumerics (Applied Maths, USA) programs.

Results. According to the results of the research, it was found that all specimens belong to the same subgenus *Francisella tularensis* subsp. *holarctica*. Within this subtype, three distinct genotypes were conventionally named according to the genotypes of other countries: Russian-Azerbaijani (5 samples), Czechoslovakia (1 sample) and European (14 samples) cluster.

Conclusions. All specimens refer to *Francisella tularensis* subsp. *holarctica*, which coincides with the OIE and WHO data for the distribution of various subtypes of *F. tularensis* throughout the globe.

The most commonly encountered genotype related to the "European" cluster. Tularemia outbreaks and isolation of the causative agent with this genotype were registered in 1996, 1998–2000, 2003, 2005, 2006, 2008, 2010, 2011 and 2016 in 11 regions of Ukraine. It can be assumed that this genotype is typical for the territory both for Ukraine and for many European countries. The sample, which was attributed to the "Czechoslovak" genotype, was isolated from hay, and may have been accidentally brought to the country.

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Characterization of Ukrainian *Brucella* strains by a Bruce-ladder multiplex polymerase chain reaction assay

Mykhailenko, A.[1]; Schwarz, J.[2]; Duerr, A.[2]; Bolotin, V.[1]; Gerilovych, A.[1]

[1] National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine
[2] Bundeswehr Institute of Microbiology, Department for Bacteriology and Toxicology, Munich, Germany

Background. Brucellosis is a vicious worldwide zoonotic disease, caused by various species of the genus *Brucella*. Nowadays, Ukraine is free from the infection, but Brucellosis sporadically occurs in humans, domestic and wild animals populations (dogs, cattle, ships, wild boars, etc.). National Scientific Centre Institute of Experimental and Clinical Veterinary Medicine (NSC IECVM) has a unique collection of *Brucella* strains, isolated since 1945, which covers vast geographical parts of Ukraine. Maintenance of the collection requires revision of strains, concerning their morphological, biochemical and genetical context. In the study, we focus on the typing of collection strains isolated in the last 70 years from different regions and sources of Ukraine by PCR multiplex Bruce-ladder.

Materials and Methods. *Brucella* strains were previously isolated from cattle and pigs in Odessa, Lugansk, Kherson, Kharkiv, Poltava and Chernihiv regions in the period of 1945–1975. In addition two vaccine strains (*B. abortus* S19 and *B. abortus* 54) and one reference *B. abortus* strain 99 were used as positive control. All strains were defined as *B. abortus* (n = 8) and *B. suis* (n = 10) by phenotyping. Since isolation the samples have been constantly recultivated or kept as lyophilized samples. The species affiliation of inactivated strains was determined using the Bruce-ladder multiplex PCR assay (Lopez Goñi et al., 2008).

Results. According to our study, all the *B. abortus* field strains as well as reference strain *B. abortus* 99 reveal the following amplification products, using Bruce-ladder PCR approach: 152, 450, 587, 794 and 1,682 bp, whereas the amplified 587 bp product that corresponds to *ery*C gene was not observed in two vaccine strains *B. abortus* S19 and *B. abortus* 54. S19 strain, isolated in 2001 has shown an absence of 1,682 bp PCR product (*wboA* gene, that responds for the LPS synthesis). The *B. suis* strains showed amplification products of 152, 272, 450, 587, 794, 1,071 and 1,682 bp as expected.

Conclusions. Bruce-ladder PCR is useful in differentiating all *Brucella* species which enable us to use the collection efficiently, reveal possible unique strains and provide the following genotyping in order to obtain data for advanced understanding of the brucellosis epidemiology in Ukraine.

Validation of qPCR method for detection of *Bacillus anthracis* pXO2 plasmid at NSC IECVM

O. Biloivan[1]; A. Duerr[2]; J. Schwarz[2]; B. Stegniy[1]; O. Solodiankin[1]; A. Gerilovych[1]

[1] National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine
[2] Bundeswehr Institute of Microbiology, Department for Bacteriology and Toxicology, Munich, Germany

Introduction. Anthrax is particularly dangerous zoonotic disease caused by *Bacillus anthracis*. It is non-motile, gram-positive and facultative anaerobic rod. Depending on route of infection, it may cause cutaneous, gastro-intestinal and pulmonary anthrax. The last one is caused when anthrax spores are inhaled into lungs from environment, where they can persist for decades before penetration into animal or human body, where they turn into vegetative form and spread with blood stream. But the most crucial factor that stipulates virulence of *B. anthracis* is its ability to form capsule inside the host organism. This capsule protects bacterial cells from lysis by host's mononuclear phagocyte system and encoded by genes of pXO2 plasmid, which, together with chromosome and toxigenic pXO1 plasmid form anthrax genome. Together with classical bacteriological and serological tests, conventional and quantitative PCR (qPCR) are commonly used for rapid diagnostics of anthrax. Therefore, it is essential

to provide accurate and correct results of diagnostic assays to make it maximally specific, robust, repeatable and sensitive.

Objective. To validate qPCR method for detection of *capC* genetic marker of *Bacillus anthracis* pXO2 plasmid and to adjust this assay for processing at the laboratory of molecular diagnostics in National Scientific Center Institute of Experimental and Clinical Veterinary Medicine (NSC IECVM, Kharkiv, Ukraine).

Materials and methods. Specific *capC* primers and TaqMan *capC* probe manufactured by Molbiol (Germany), as well as AmpliTaq Gold reagents by Applied Biosystems (USA) were used for validation assays. All qPCR tests were carried out using ABI Fast 7500 Real-time PCR system. Statistical calculations were conducted using standard Microsoft Office software (MS Excel).

Results. The first stage of validation procedure was to determine optimal concentration of template DNA for assays. For this purpose, cloned *capC* plasmid DNA was cleaned up from vector using standard techniques, and number of copies in 5 μl of template was calculated based on its concentration measured with Nanodrop. Dilution series from 10^9 copies to 1 copy of DNA were prepared and 10^3 copies dilution with CT value of 30 was used as template for further assays. The robustness of qPCR was adjusted by optimization of amplification parameters (annealing temperature), and concentration of reaction components ($MgCl_2$, primers, probe and *Taq* polymerase). The next step was to determine precision and repeatability by evaluation of variation within the experiment (inter-assay variability) and between several independent experiments (inter-assay variability). Analytical sensitivity of the method was defined using probit analysis and linearity test. Probit analysis with serial dilutions of positive control with 5 replicates per dilution was carried out to define the 95% limit of detection (LOD), and, to determine the correlation of CT value with amount of DNA in template, the linearity test was conducted. To define the ability to detect sequence of interest (diagnostic sensitivity), we tested mixed panel of *Bacillus anthracis* strains. As the result, *capC* marker could be detected in all tested strains. To find out the specificity of our assay, we also tested various strains of *B. cereus, B. thuringiensis, B. mycoides* and *B. globigii* (potential cross-reacting organisms) as well as DNA samples of various pathogenic bacteria and viruses which cause similar clinical symptoms as anthrax (differential diagnosis relevant organisms). As the result, only four out of six strains of *Bacillus cereus* were positive with different CT values, which were expected to contain *capC* genetic marker.

Conclusions. All conducted assays proved that qPCR test which we use for detection of *capC* genetic marker of *Bacillus anthracis* pXO2 plasmid is specific and trustable. In addition, this validation procedure allowed to make this test more robust, repeatable and sensitive. This method will be adjusted in the laboratory of molecular diagnostics at NSC IECVM for diagnostical detection of anthrax in environmental samples.

LIST OF PARTICIPANTS

No	Name	Institution	E-mail
1	Aleksandra Inic-Kanada	Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria	aleksandra.inic-kanada@meduniwien.ac.at
2	Alison Favaroni	Friedrich-Loeffler-Institut, Jena, Germany	alison.favaroni@fli.de
3	Anastasiia Mykhailenko	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	anst.mykhailenko@gmail.com
4	Andreas Pospischil	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	apos@vetpath.uzh.ch
5	Anne Ammerdorffer	TubaScan/ VU University Medical Center Amsterdam, The Netherlands	a.ammerdorffer@vumc.nl
6	Anton Gerilovych	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	antger2011@gmail.com
7	Barbara Prähauser	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	barbara.praehauser@uzh.ch
8	Barbara Susanne Sixt	The Laboratory for Molecular Infection Medicine Sweden, Umeå University, Sweden	barbara.sixt@umu.se
9	Borys Stegniy	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	admin@vet.kharkov.ua
10	Bryan Markey	University College Dublin, Ireland	bryan.markey@ucd.ie
11	Carlos Montbau	HIPRA, Amer, Spain	roger.guix@hipra.com
12	Cheng He	Key Lab of Animal Epidemiology and Zoonosis, Agricultural University, Beijing, China	hecheng@cau.edu.cn
13	Chloe Elizabeth Manning	University of Southampton, England	c.e.manning@soton.ac.uk
14	Christiane Schnee	Friedrich-Loeffler-Institut, Jena, Germany	christiane.schnee@fli.de
15	Christine Unterweger	University Clinic for Swine, Vetmeduni, Vienna, Austria	christine.unterweger@vetmeduni.ac.at
16	Colette Elizabeth O'Neill	University of Southampton, England	con2r10@soton.ac.uk
17	Daisy Vanrompay	UGent , The Netherlands	daisy.vanrompay@ugent.be
18	Danijela Horvatek Tomic	Faculty of Veterinary Medicine Zagreb, Crotia	horvatek@vef.hr
19	David Longbottom	Moredun Research Institute, Scotland	david.longbottom@moredun.ac.uk
20	Delia Onorini	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	delia.onorini@studio.unibo.it

No	Name	Institution	E-mail
21	Elisabeth Liebler-Tenorio	Friedrich-Loeffler-Institut, Jena, Germany	elisabeth.liebler-tenorio@fli.de
22	Emma Cousins	University of Southampton, England	e.cousins@soton.ac.uk
23	Estella Prukner-Radovcic	Faculty of Veterinary Medicine Zagreb, Crotia	prukner@vef.hr
24	Firuza Bayramova	Lausanne University Hospital and Lausanne University, Lausanne, Switzerland	firuza.bayramova@chuv.ch
25	Ganna Orekhova	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	orekhovaganna@gmail.com
26	Gaston Caspe	Moredun Research Institute, Scotland	gaston.caspe@moredun.ac.uk
27	Ian Clarke	University of Southampton, England	inc@soton.ac.uk
28	Ihor Kotsiumbas	State Research Control Institute of Veterinary Medicinal Products and Feed Additives, Lviv, Ukraine	dir@scivp.lviv.ua
29	Janine Fritschi	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	janine.fritschi@uzh.ch
30	Jasmin Kuratli	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	jasmin.kuratli@uzh.ch
31	Jizhang Zhou	Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China	zhoujizhang@caas.cn
32	Juliia Krivosheii	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	yuliettkrivoshei@gmail.com
33	Karine Laroucau	ANSES, Paris, France	karine.laroucau@anses.fr
34	Katharina Tenorio	Georg-August Universität, Göttingen, Germany	kathi.tenorio@web.de
35	Kensuke Shima	University of Lübeck/Department of Infectious Diseases and Microbiology, Germany	kensuke.shima@uksh.de
36	Krzysztof Niemczuk	National Veterinary Research Institute, Pulawy, Poland	kniem@piwet.pulawy.pl
37	Laura del Rio Alonso	Veterinary Faculty, University of Murcia, Spain	laurario@um.es
38	Liudmila Vladimirovna Rubanik	Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, Republic of Belarus	rubaniklv@tut.by
39	Maria Rosa Caro	Veterinary Faculty, University of Murcia, Spain	mrcaro@um.es
40	Marloes Heijne	Wageningen Bioveterinary Research, Lelystad, The Netherlands	marloes.heijne@wur.nl
41	Maryna Stegniy	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	admin@vet.kharkov.ua
42	Md Mominul Islam	University of the Sunshine Coast, Australia	mominul.islam@research.usc.edu.au

No	Name	Institution	E-mail
43	Monika Szymańska-Czerwińska	National Veterinary Research Institute, Pulawy, Poland	monika.szymanska@piwet.pulawy.pl
44	Morag Livingstone	Moredun Research Institute, Scotland	morag.livingstone@moredun.ac.uk
45	Nadine Käppeli	Institute for Food Safety and Hygiene, Vetsuisse Faculty, Zurich, Switzerland	nadine.kaeppeli@uzh.ch
46	Nicole Borel	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	n.borel@access.uzh.ch
47	Oksana Zlenko	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	oksana.ceratium@gmail.com
48	Oleksandr Biloivan	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	silverscreen91@gmail.com
49	Olexii Solodiankin	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	olexii.solod@gmail.com
50	Peter Marsh	University of Southampton, England	p.marsh@soton.ac.uk
51	Peter Timms	University of Sunshine Coast, Queensland, Australia	ptimms@usc.edu.au
52	Prisca Mattmann	Department of Poultry Diseases, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Switzerland	prisca.mattmann@uzh.ch
53	Rachel Jane Skilton	University of Southampton, England	r.j.skilton@soton.ac.uk
54	Robert V. Schoborg	Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA	schoborg@mail.etsu.edu
55	Sara Rigamonti	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, National Reference Laboratory for Chlamydioses, Italia	sara.rigamonti@izsler.it
56	Servaas Morré	VU University Medical Center, Amsterdam, The Netherlands	samorretravel@yahoo.co.uk
57	Simone Magnino	Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, National Reference Laboratory for Chlamydioses, Italia	simone.magnino@izsler.it
58	Teresa Calvo González-Valerio	HIPRA, Amer, Spain	teresa.calvo@hipra.com
59	Theresa Pesch	Institute for Veterinary Pathology, Vetuisse Faculty, Zurich, Switzerland	theresa.pesch@uzh.ch
60	Vitaliy Bolotin	National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine	vbolotin@hotmail.de
61	Yvonne Pannekoek	Dept. of Medical Microbiology, Amsterdam UMC, The Netherlands	y.pannekoek@amc.uva.nl