

**49. Jahrestagung der Österreichischen Gesellschaft
für Tropenmedizin, Parasitologie und Migrationsmedizin**

***49th Annual Meeting of the Austrian Society
of Tropical Medicine, Parasitology and Migration Medicine***

“Breakthroughs”



**Programm
*Programme***



**Kurzfassungen
*Abstracts***

Natural History Museum
Vienna, Austria
12. – 14. November 2015

www.ogtpm.at

Veranstaltungsort/ *Meeting Venue*:

**Naturhistorisches Museum Wien
Natural History Museum Vienna
Burgring 7, 1010 Wien**

Dear Colleagues and Friends,

Hereby I would like to cordially invite you to the 49th Annual Meeting of the Austrian Society of Tropical Medicine, Parasitology and Migration Medicine, which will be held from November 12-14, 2015 at the Natural History Museum in Vienna. This year's conference is entitled "Breakthroughs" and again we have tried to compile an exciting programme, embracing the manifold activities of our society in the areas of tropical medicine, parasitology and migration medicine.



The scientific programme will cover a diversity of topics, including basic, translational and clinical research, as well as case reports and skill enhancements.

Also this year we have been able to invite renowned researchers from Austria and abroad to present their cutting-edge research. We hope that the variety of topics provide a "healthy mix" of molecular biology, epidemiology and clinical research and will attract participants from a wide range of fields. There is scope for submission of presentations in any aspect of tropical medicine, parasitology and migration medicine.

Besides oral presentations we will again also have a chaired poster session giving the authors the opportunity to present and discuss their work.

We would like to particularly encourage also young scientists to attend the meeting, competitive junior and poster awards will again be provided.

On this occasion I would like to express my sincere thanks to our sponsors, who support us once again in the organisation and the funding of the meeting.

I am looking forward to a thrilling, informative and rewarding conference and to seeing you all in Vienna!

On behalf of the conference committee and the board of the ÖGTPM,

Sincerely yours,

Julia Walochnik

President of the ÖGTPM

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THURSDAY, NOVEMBER 12th

08:30 – 09:00 Registration

09:00 – 09:10 Welcome and Introduction

NOVEL VACCINES

Chair: Ursula Wiedermann & Peter Kreamsner

09:10 – 09:55 **KEYNOTE LECTURE**

Benjamin Mordmüller: Malaria vaccine

09:55 – 10:40 **KEYNOTE LECTURE**

Maxime Selidji Agnandji: Ebola vaccine

10:40 – 11:10 *Coffee break*

TROPICAL MEDICINE

Chairs: Rosemarie Moser & Martin Haditsch

11:10 – 11:30 **Martin Haditsch**: Smallpox vaccine (...as an example of bio-preparedness)

11:30 – 11:45 **Matthias Wittrock**: Is classification of Leprosy as an NTD a game changer?

11:45 – 12:00 **Felix Lötsch, Ghyslain Mombo-Ngoma, Mirjam Groger, Ayôla A Adegnika, Selidji T Agnandji, Herbert Auer, Michael Ramharter**: Epidemiology of Cystic Echinococcosis in the Province of Moyen-Ogooué, Gabon, Central Africa

12:00 – 12:15 **Felix Lötsch, Judith Naderer, Mirjam Groger, Herbert Auer, Klaus Kaczirek, Fredrik Waneck, Michael Ramharter**: Intra-cystic drug concentration of albendazole and its active metabolite albendazole-sulphoxide in human cystic echinococcosis: A systematic review and analysis of individual patient data

13:00 – 14:00 **LUNCH SYMPOSIUM „SELTENE PARASITOLEN“ (in German):**
PARASITOLEN DES ZNS
(Organized by INSTAND & ÖQUASTA)

Chairs: Horst Aspöck & Klaus Janitschke

Infektionen durch *Balamuthia mandrillaris* (Albrecht Kiderlen)

***Acanthamoeba*-Enzephalitis (Julia Walochnik)**

Helminthen-Infektionen des ZNS (Herbert Auer)

MOLECULAR AND EXPERIMENTAL PARASITOLOGY

Chairs: Irma Schabussova & Michael Duchêne

14:30 – 15:00 **KEYNOTE LECTURE**

Graham Warren: Studying Golgi biogenesis using *Trypanosoma brucei*

15:00 – 15:15 ***Erik Küng, Jacek Pietrzak, Christoph Klaus, Julia Walochnik:**
Octenidine dihydrochloride killing *Trichomonas vaginalis*

15:15 – 15:30 **David Leitsch, Samuel Rout, Adrian Hehl, Norbert Müller:** *Giardia lamblia*
thioredoxin reductase: a well-known enzyme with unusual characteristics

15:30 – 15:45 **Katharina Paschinger, Shi Yan, Iain B. H. Wilson:** Comparative Nematode
Glycomics

16:00 – 16:30 *Coffee break*

NYP “PARASITES AS HOSTS”

Chairs: Ursula Fürnkranz & Hans-Peter Führer

16:30 – 17:00 **KEYNOTE LECTURE**

Silvia Cervero-Aragó, S. Rodríguez-Martínez, A. Puertas-Bennasar, R. Sommer, R. Araujo: Microbe-host interaction affects the resistance of water-borne pathogen *Legionella pneumophila* to disinfectants

17:00 – 17:15 **Ursula Fürnkranz, Julia Walochnik, Birgit Henrich:** Long-time co-culture of *Trichomonas vaginalis* and *Mycoplasma hominis* leads to a change in the tetracycline-resistance of *M. hominis*

17:15 – 17:30 ***Elisabeth Dietersdorfer, Sílvia Cervero-Aragó, Regina Sommer, Alexander K. Kirschner, Julia Walochnik:** Optimized methods for *L. pneumophila* release from its *Acanthamoeba* hosts

17:30-19: 30 GET TOGETHER (NHM)

FRIDAY, NOVEMBER 13th

08:30 – 09:00 Registration

09:00 – 09:45 **KEYNOTE LECTURE**

Chair: Erich Schmutzhard

Sabine Rüsç-Gerdes: Tuberculosis – a new threat in Europe?

09:45 – 10:15 *Coffee break*

VETERINARY PARASITOLOGY & ZOOSES

Chairs: Anja Joachim & Georg Duscher

10:15 – 10:30 ***Gumpinger P., Konecny R., Kitaka N., Waidbacher H.:** Parasite community richness of fish from Lake Baringo, Kenya

10:30 – 10:45 **W. Glawischnig, C. Schleicher, K. Schöpf:** Prevalence on sylvatic *Trichinella* spp. in red foxes (*Vulpes vulpes*) in Tyrol and Vorarlberg

11:00 – 11:15 ***Natascha Leitner, Laurin Schwarzmann, Carina Zित्रa, Nicola Palmieri, Barbara Eigner, Domenico Otranto, Walter Glawischnig, Hans-Peter Fuehrer:** Larvae causing nasopharyngeal myiasis of red deer (*Cervus elaphus*) from Vorarlberg and Tyrol – morphological and molecular assessment

11:15 – 11:30 ***Nadine Hohensee, Helmut Sattmann, Christoph Hörweg, Julia Walochnik:** Digenean trematodes in freshwater snails in the surroundings of Vienna with a focus on species involved in human infections

11:30 – 11:45 ***Christian Husch, Helmut Sattmann, Christoph Hörweg, Julia Walochnik:** Diversity of *Fascioloides magna* and *Fasciola hepatica* in Austria based on their genes and antigens

11:45 – 12:00 **Franz Jirsa, Florian Krennmayr, Christa Fellner – Frank:** Parasites of white-eye bream *Ballerus sapa* (Pallas, 1814), perch *Perca fluviatilis* L, 1758 and other fish species from the Austrian Danube

12:30 – 13:30 **LUNCH SYMPOSIUM II: HIV UND OPPORTUNISTEN** (in German)

Chairs: Wolfgang Graninger & Julia Walochnik

HIV-Therapie (Armin Rieger)

Opportunisten (Heimo Lagler)

VEHICLES AND VECTORS

Chairs: Angelika Wagner & Christoph Hörweg

14:00 – 14:15 ***Verena Mündler, Iveta Häfeli, Ivo Steinmetz, Mamadou Sawadogo, Julia Walochnik:** *Acanthamoeba* spp. as possible host organisms for the pathogen *Burkholderia pseudomallei*

- 14:15 – 14:30 ***Adnan Hodžić, Hans-Peter Fuehrer, Georg Gerhard Duscher**: Spotted fever group rickettsiae in ticks in Bosnia and Herzegovina
- 14:30 – 14:45 **Adelheid G. Obwaller, Mehmet Karakus, Wolfgang Poepl, Seray Toz, Yusuf Ozbel, Horst Aspöck, Julia Walochnik**: Evidence for stable endemic sandfly populations in the light of migration streams into Austria
- 14:45 – 15:00 ***Carina Zित्रa, Eva Flechl, Barbara Eigner, Adelheid Obwaller, Heidemarie Rossiter, Karin Lebl, Thomas Zechmeister, Johann Waringer, Hans-Peter Fuehrer**: DNA-Barcoding of the Austrian mosquito species (Diptera: Culicidae) inventory
- 15:00 – 15:15 ***Eva Flechl, Carina Zित्रa, Michael Kothmayer, Simon Vitecek, Heidemarie Rossiter, Thomas Zechmeister, Hans-Peter Fuehrer**: Ecological characterization and molecular differentiation of the *Culex pipiens* complex in Eastern Austria
- 15:30 – 16:00 *Coffee break*

POSTER SESSION

Chairs: Mirjana Drinic & David Leitsch

16:00 – 17:00 Guided poster session in the lecture hall

17:00 – 18:00 **GENERALVERSAMMLUNG (lecture hall)**

EVENING EVENT

Gala Dinner (NHM Upper Hall)

Honorary Membership LAUDATIO PROF. HORST ASPÖCK

HANDING OVER of the JUNIOR-AWARD (*courtesy of ÖGTPM*)

HANDING OVER of the POSTER-PRIZE (*courtesy of ÖGTPM*)

SATURDAY, NOVEMBER 14th

FORTBILDUNG ÄRZTE/APOTHEKER (in German language)

08:30 – 09:00 Registrierung

09:00 – 11:00 REISEMEDIZINISCHES UPDATE

Chairs: Ursula Wiedermann und Herwig Kollaritsch

09:00 – 09:20 **Stefan Winkler**: Kranke Reiseheimkehrer: Eine diagnostische Herausforderung?

09:20 – 09:40 **Angelika Wagner**: International Health Regulations: Was ist neu?

09:40 – 10:00 **Bernhard Haberfellner**: Damit der schönste Urlaub nicht der letzte wird: Reisetauglichkeit bei Grundkrankheiten

10:00 – 10:20 **Ursula Wiedermann**: Impfungen beim immunsupprimierten Reisenden

10:20 – 10:40 **Herwig Kollaritsch**: Aktuelle Weltseuchenlage

10:40 – 11:00 *Kaffeepause*

11:00 – 12:00 **REISE- & TROPENMEDIZIN: QUIZ mit VOTING** (*mit Unterstützung durch MSD*) **Herwig Kollaritsch**

Ebola vaccine

Maxime Selidji Agnandji

Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon

Helminth infections of the central nervous system

Herbert Auer

Med. Parasitology, Institute of Specific Prophylaxis and Tropical Medicine, Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Austria
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The spectrum of helminths affecting the human central nervous system comprises trematode, cestode and nematode species. The lecture gives an overview about the most important species and describes synoptically the epidemiology and nosology of the diseases caused by them. Among these, particular attention is devoted to cerebral echinococcosis, neurocysticercosis and cerebral coenurosis as well as neurotoxocarosis. Own experiences with the laboratory diagnostic procedures of these helminthic infections are presented.

Microbe-host interaction affects the resistance of water-borne pathogen *Legionella pneumophila* to disinfectants

Silvia Cervero-Aragó, S. Rodríguez-Martínez, A. Puertas-Bennasar, R. Sommer, R. Araujo

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The interaction of bacteria and protists is considered to be one of the oldest prey–predator interactions in nature. The predation by phagocytic protists causes a high bacterial mortality and represents a significant driving force of the genetic and functional structure of bacterial communities. Thus, many bacteria have evolved developing a wide variety of anti-predator strategies. One example of this is *Legionella pneumophila*, Gram-negative bacteria ubiquitous in natural and man-made aquatic environments and the etiologic agent of Legionnaires' disease. Human alveolar macrophages act, in this case, as accidental *Legionella* hosts after the inhalation of small aerosols from contaminated water devices such as cooling towers, showerheads and faucets. The proliferation of the bacteria in water systems is closely related to the interaction with protozoa, which not only provide nutrients for the intracellular legionellae, but also represent a shelter against harsh environmental conditions and disinfection techniques. The aim of the current study was to determine if the interaction of protozoa specifically *Acanthamoeba* and *L. pneumophila*, reduces the effectiveness of common drinking water disinfection techniques such as chlorine, temperature and UV irradiation.

Inactivation results of the *Legionella*-amoeba association reduced the effectiveness of the treatments applied. Remarkably, at the lowest free chlorine concentration, 0.5 mg L⁻¹, as well as at the lowest temperatures, 50°C and 55°C, the influence of the *Legionella*-amoeba associate state was the strongest compared to the free *Legionella* state. Regarding UV irradiation, results showed that the association of *L. pneumophila* with free-living amoebae decreases its effectiveness against the bacteria in a range of 1.5 to 2 fold. The often-underestimated association established between the different microorganisms in water systems can modify the effectiveness of microbicidal treatments and promote *Legionella* recolonization. This applies particularly to the proximal areas of the systems (close to the tap), where lower free chlorine concentrations and lower temperatures are commonly observed and has to be considered to protect human health.

Optimized methods for *L. pneumophila* release from its *Acanthamoeba* hosts

Elisabeth Dietersdorfer¹, Silvia Cervero-Arago^{2,3}, Regina Sommer^{2,3}, Alexander K. Kirschner^{2,3}, Julia Walochnik¹

¹ Department of Medical Parasitology, Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna.

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Acanthamoebae serve as vehicles and hosts for *Legionella pneumophila*, among other pathogenic microorganisms. To study this ecological relationship, co-culture and infection models came into the focus of interest in the past years. However, there is a lack of data regarding the effectiveness of the different methods to release the intracellular bacteria from their amoebal hosts. The aim of this study was to evaluate the impact of the respective methods used to release intracellular *L. pneumophila* cells on the culturability of the bacteria. Furthermore, the standard method ISO 11731:1998 was evaluated for its suitability to quantify intracellular *L. pneumophila*. Results showed that the effectiveness of the eight release treatments applied on *L. pneumophila* and *Acanthamoeba* strains in a free-living state varied between bacterial strains. Regarding the release treatments on the co-cultures of *L. pneumophila* and *Acanthamoeba* an enhanced survival was observed for both microorganisms. The passage through a needle (21G, 27G) as well as centrifugation at 10,000 × g showed the highest bacterial counts when releasing the bacteria. Regarding the ISO 11731:1998 method, a reduced bacterial recovery rate was observed for one of the co-cultured bacteria strains in comparison to the free-living state, whereas the other strain showed similar recoveries between both states.

Mechanical release treatments were the most effective methods providing a bacterial release without interfering chemical remainings for further experimental steps in the study of intracellular bacteria. The recovery of *L. pneumophila* from water systems can be underestimated if protozoal membranes are not disrupted. Therefore a mechanical treatment step should be considered in the ISO 11731:1998.

Pathogenesis of leprosy: an insight in the role of B-lymphocytes and plasma cells

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Leprosy, also known as Hansen's Disease, is a chronic infectious disease of the skin and peripheral nerves, caused by *Mycobacterium leprae*. The pathogenesis of the disease is still not fully understood. Several studies have been performed on the appearance of T-cells in leprosy, but there are still only few reports about the role of B-cells in active leprosy lesions in different spectral forms of the disease. In the last years, studies performed on other mycobacterial diseases, especially tuberculosis, could show that B-cells are of great importance for the granulomatous immune response. Therefore their influence on the course of the disease and the outcome of the therapy is larger than anticipated earlier.

The purpose of this anonymous, retrospective study was to demonstrate the presence of B-cells and plasma cells in skin lesions of all the leprosy forms according to the Ridley-Jopling classification, including the leprosy reactions, to gain a better insight on the role of B-cells and plasma cells in the immunopathogenesis of leprosy.

72 leprosy skin biopsy samples from Manaus/Brazil as well as 13 from Genoa/Italy were immunohistochemically stained and analyzed by light-optical microscopy, using CD20 as a marker for B-cells and CD138 as a marker for plasma cells. For comparison, 13 skin biopsy samples of cutaneous tuberculosis and atypical mycobacterial infections from Rome were examined. The results were statistically analyzed, with a level of significance of $p \leq 0.05$.

The presence of both B- and plasma cells could be demonstrated for the first time in all spectral forms of the disease (I, TT, BT, BB, BL, LL) as well as in the leprosy reactions.

Large amounts of B-cells were found in TT and type 1 reaction, thus contradicting findings from earlier studies, which proposed an increase of the B-cell count from the tuberculoid to the lepromatous pole. Our results indicate that B-cells are taking part in the formation of granulomes in leprosy.

Plasma cell counts were considerably higher at the lepromatous pole than at the tuberculoid pole. However, the occurrence of plasma cells at the tuberculoid pole indicates that they also play a role in the pathogenesis of the paucibacillary forms.

Ecological characterization and molecular differentiation of the *Culex pipiens* complex in Eastern Austria

**Eva Flechl¹, Carina Zित्रा¹, Michael Kothmayer¹, Simon Vitecek², Heidemarie Rossiter³
Zechmeister Thomas⁴, Hans-Peter Fuehrer¹**

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Species of the *Culex pipiens* complex, better known as common house mosquitoes, are morphologically cryptic (i.e., hardly identifiable due to their high morphological similarity) but differ extensively in behaviour, physiology and also have different vector capacities. The complex is considered to consist of 8 taxa worldwide, of which 3 are reported from Central Europe. The nominal species of the *Culex pipiens* complex, *Culex (Culex) pipiens* L. comprises of two genetically and ecologically distinct forms: *Culex pipiens* f. *pipiens* is mainly ornithophilic, anautogenous and eurygamous, whereas the form *molestus* is mammophilic, autogenous and stenogamous. Both forms are known to hybridize in areas of coexistence, potentially resulting in bridge vector populations with mixed feeding preferences. *Culex torrentium* is a morphologically highly similar sister taxon of *Culex pipiens* L. and exhibits almost the same bionomic characteristics.

Mosquitoes were collected from April to October 2014 at 27 permanent sampling sites, distributed across Vienna, Burgenland and Lower Austria. Members of the *Culex pipiens* complex were sampled biweekly for 24 hours, using specific mosquito traps equipped with carbon dioxide as an attractant. Each of the 1,476 sampled mosquitoes was homogenized and genetically identified using either the CQ11 locus to distinguish between *Culex pipiens* f. *molestus* and *Cx. pipiens* f. *pipiens* or an intron polymorphism in the *ace-2* gene to distinguish between the *Cx. pipiens* complex and *Cx. torrentium* as well as to identify hybrid populations.

In this study we aim to assess the differential presence and distribution of *Culex pipiens* complex taxa in Eastern Austria and additionally present terrestrial environmental factors determining seasonal and spatial distribution patterns of this species complex.

This research was funded by the ERA-Net BiodivERsA, with the national funders FWF I-1437, ANR-13-EBID-0007-01 and DFG BiodivERsA KL 2087/6-1 as part of the 2012-13 BiodivERsA call for research proposals.

Long-time co-culture of *Trichomonas vaginalis* and *Mycoplasma hominis* leads to a change in the tetracycline-resistance of *M. hominis*

Ursula Fürnkranz^{1,2}, Julia Walochnik¹, Birgit Henrich²

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2 Institute for medical Microbiology and Hospital Hygiene, Heinrich-Heine-University Düsseldorf, Germany

Trichomonas vaginalis and *Mycoplasma hominis* have been described to undergo a close relationship in the human body. In the present work, this relationship has successfully been confirmed *in vitro* using several clinical isolates of the parasites and several isolates of the bacterium.

In three of these isolates of *M. hominis*, a transposon was discovered, carrying the *tetM*-gene and thus causing high resistance to tetracycline. The influence of passaging of *M. hominis* through *T. vaginalis* on the tetracycline-resistance was investigated during the course of co-culturing.

First results of the continuous examination of the MIC (minimal inhibitory concentration) against tetracycline, as well as the survey of the presence of the transposon and the *tetM*-gene using a novel designed qPCR indicate, that those strains formerly carrying the *tetM*-transposon, loose it in the time-course of co-culture. This phenomenon was never described before and did not take place in *tetM*- positive *M. hominis* strains after passaging through a human cell line such as HeLa cells.

Prevalence on sylvatic *Trichinella* spp. in red foxes (*Vulpes vulpes*) in Tyrol and Vorarlberg

W. Glawischnig¹, C. Schleicher², K. Schöpf¹

¹Institute for Veterinary Disease Control Innsbruck, Austrian Agency for Health and Food Safety (AGES)

²Data, Statistics and Risk Assessment Graz, Austrian Agency for Health and Food Safety (AGES)

Sylvatic Trichinellosis affects carnivores with cannibalistic and scavenger behavior. In most regions of the EU the red fox (*Vulpes vulpes*) is the specific reservoir for this disease. At the same time the red fox is the main reservoir for *T. britovi*. In Austrian wildlife *T. britovi* is the predominant circulating species. Findings of positive foxes are mainly located in the Western and Southern regions of the country where also sporadic cases in badgers are documented.

Within a recent study on the occurrence of zoonotical parasites in hunted red foxes originating from the two Alpine provinces of Austria, we examined muscle samples for the presence of *Trichinella* larvae. Based on a statistical sample plan, 746 hunted carcasses from the province Tyrol (n=415) and Vorarlberg (n=331) were sampled during winter season 2013/14 and 2014/15. Data collection included the recording of sex, age, weight, geographic origin of the samples and body condition. *Trichinella* examination was done by magnetic stirrer method for pooled samples. Muscle tissue (5 gram) of the front leg was digested and positive pooled samples were further individually tested in order to identify the infected animal. To determine the genotype of the recovered *Trichinella* larvae species differentiation was performed by multiplex PCR.

Trichinella larvae were observed in a total of 28 examined animals (3.2%, with 95%-confidence interval [1.5%, 5.2%]). In Vorarlberg an apparent prevalence of 6.3% [3.9%, 9.6%] was observed, in Tyrol apparent prevalence was 1.7% [0.6%, 3.5%]. Logistic regression indicates that the geographic origin of samples (province) and the age category have a significant effect on the likelihood of the occurrence of *Trichinella* larvae. More specifically, red foxes originating from Vorarlberg have a significantly higher probability to be *Trichinella* positive compared to foxes from Tyrol (odds ratio = 4.1). Adult foxes are more likely infected compared to juvenile foxes (odds ratio = 2.7). Species identification from all positive samples revealed *T. britovi* as the only infectious parasite species.

The present study confirmed that *T. britovi* is prevalent in wild carnivores in Austria. Foxes act as a wildlife reservoir for this species. In comparison to a previous study our results confirmed the prevalence found in foxes originating from Tyrol but revealed a 4 times higher prevalence in foxes from Vorarlberg.

Parasite community richness of fish from Lake Baringo, Kenya

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A fish parasitological survey was carried out at Lake Baringo, Kenya from March to April 2015 to study the presence of endoparasites of 4 fish species occurring in the lake. A total of 101 specimens of *Oreochromis niloticus baringoensis*, *Protopterus aethiopicus*, *Barbus intermedius australis* and *Clarias gariepinus* were examined using conventional parasitological techniques. The focus of the investigation was on intestinal helminths of the systematic groups Digenea, Cestoda, Nematoda and Acanthocephala. In addition to the epidemiological parameters prevalence, mean intensity and abundance, parasite species richness and diversity for the most dominant parasites were calculated. The endoparasite fauna was dominated by larval cestodes, metacercariae of digenean trematodes and larval nematodes. Digenean metacercariae of the genus *Clinostomum* sp. and larval nematodes of the genus *Contracaecum* sp. were recorded, which could represent a risk for human infections. Results were compared with parasite data from other lakes in East Africa and discussed in relation to the life cycles of the parasites and also in respect to the changing ecological conditions of Lake Baringo.

Damit der schönste Urlaub nicht der letzte wird: Reisetauglichkeit bei Grundkrankheiten

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Smallpox vaccine (...as an example of bio-preparedness)

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The smallpox virus affected mankind for about 12000 years. It is an Orthopox virus which caused devastating epidemics: some 300 – 500 Mio. people died due to smallpox in the 20th century. In addition to natural epidemics the smallpox virus was also used as a biological warfare (BW) agent for centuries. Due to the dynamics this disease always had a major impact on the society and economy affected.

If we crosscheck impact and incidence it is clear that high impact infectious diseases (will) create the highest need for counterfight weapons. Examples of the recent past and the present are SARS, pandemic flu, MERS-CoV, Influenza A/H5N1 and Ebola. Actually, out of high impact events only a minimal part is vaccine preventable.

Global mandatory smallpox vaccination extinguished the disease by 26th of October 1979 which was a huge success considering that this virus is a highly contagious (R_0 : 3 - 6) as well as a very dangerous agent (CFR about 30%). Vaccination was stopped and global immunity began to decrease. Smallpox shifted to the very center of close monitorings by the western as well as by the eastern hemisphere, since reoccurrence might be due to natural causes (like mutation) or to accidental (from permafrost or neglected lab samples) as well as intentional (bio-weapon) release. Furthermore in recent years scientists were able to assemble pathogens up to a million of base pairs (BP) by de novo synthesis using datapools also published in the internet (which provide information of the composition of the smallpox virus as well). Due to waning immunity weaponized poxviruses as biological warfare agents become increasingly attractive nowadays.

Whether or not smallpox could become a biological threat most likely depends on the outcome of a global discussion whether the increasing risk justifies the aim to re-establish global immunity. Apart from costs and feasibility this decision is mainly influenced by the likelihood of (severe) adverse events: the traditional smallpox vaccine showed substantial side effects with SAE of 1:1 000 and vaccine induced deaths of 1:1 000 000. To meet the needs of safety a Scandinavian company recently launched a new live attenuated non-replicating smallpox vaccine. Since this product provides solid immunity without the risk of SAEs (not even in immunocompromised persons) it could help to raise immunity in a broad range thus decreasing the risk of a new epidemic no matter whether due to mutation, accidental or intentional release of the smallpox virus.

Take home messages:

- Viable Variola viruses are not only stored in known depositories (one lab in the USA and Russia each) but might escape from the permafrost and secret labs or be released as BW agents.
- Evolution is not limited to positive aspects, only – the concepts of (mass) destruction have changed as well (including cyber-war and de-novo synthesis of BW agents).
- For uninvolved people it's merely impossible to anticipate fateful global changes as well as thoughts and actions of mentally sick persons.
- Hoping that intolerance could be balanced, overcome or neutralized by a concept of tolerance is simply stupid.
- **Old but still true: Prepare for the worst ... and hope for the best!**

Spotted fever group rickettsiae in ticks in Bosnia and Herzegovina

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Spotted fever group (SFG) rickettsiae are obligate intracellular bacteria, mainly transmitted by ticks, which act both as vectors and reservoirs for these pathogens. The severity of rickettsioses in humans depends on the species and ranges from mild to lethal illness. In Bosnia and Herzegovina, the tick fauna is very diverse, but data on the occurrence of SFG rickettsiae is lacking. Therefore, the present study aimed to investigate the occurrence of these bacteria in questing ticks by PCR and sequencing.

A great diversity of zoonotic SFG rickettsiae, namely *Rickettsia monacensis* (1.1%), *R. helvetica* (5.7%), *R. raoultii* (5.7%) and *R. slovaca* (8.0%) were detected in questing *I. ricinus* (6/30), *D. reticulatus* (10/54) and *D. marginatus* (2/3) ticks from Bosnia and Herzegovina for the first time, indicating a public health threat to humans. Although no human infections have been reported in the country yet, physicians should be aware of the presence of these tick-borne bacteria, especially when they are faced with acute febrile illnesses after tick exposure. However, large-scale studies including all geographical regions are urgently needed to determine the distribution of tick vectors and the pathogens they transmit, and to estimate the risk of infections for humans and animals in certain areas.

Digenean trematodes in freshwater snails in the surroundings of Vienna with a focus on species involved in human infections

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Cercariae of different species of bird schistosomes are known to be the causative agents of human cercarial dermatitis. Besides *Trichobilharzia* species, which are the most frequent cause for this disease, infections may also be caused by related genera. The most important final hosts are waterfowl of the family Anatidae, however, humans may be infested when the so-called furcocercariae accidentally penetrate the skin of bathing humans. The distribution of bird schistosomes is expanding and cercarial dermatitis has been recognized as an emerging disease in Europe. In contrast, echinostomosis is a foodborne disease, which can be caused by at least 20 species of 8 genera, of which the genus *Echinostoma* is the most important. The infective metacercariae encyst in freshwater snails, fish, mollusks, crustaceans and frogs and are taken up orally by the vertebrate final host. The disease is endemic to Southeast Asia due to food patterns, however, the pathogens occur worldwide and there is a general risk of infection. Detailed knowledge of the prevalence of medically important digeneans in Austria is important for a profound risk assessment. In the current project we investigate several locations in the surroundings of Vienna and collect the main intermediate hosts, *Lymnaea stagnalis* and *Radix* spp. Out of 732 collected lymnaeid snails 8% harbored echinostome cercariae, and 1% furcocercariae. An overview of the digeneans found will be presented.

Diversity of *Fascioloides magna* and *Fasciola hepatica* in Austria based on their genes and antigens

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Fascioloides magna was introduced into Europe in the 1st half of the 20th century with game animals from North America and mainly infects the liver of deer. *Fasciola hepatica* occurs in over 50 countries and infects the liver of various mammals including humans. As the main hosts are sheep and cattle, *F. hepatica* also has a great impact on meat and milk production and leads to significant economy loss annually.

The aim of this study was to investigate the genetic diversity of these flukes in Austria. For *F. hepatica*, these data are compiled for the first time in Austria. To distinguish the haplotypes the conserved mitochondrial genes *cox1* and *nad1* of 26 individuals of *F. magna*, isolated from deer from the floodplains south of Vienna, and 31 individuals of *F. hepatica*, obtained from cattle from various regions in Austria, were examined and compared with reference strains. It was shown that all 26 individuals of *F. magna* investigated are of the same haplotypes, namely COX1-Ha3 and NAD1-Ha4, respectively. These are common also in Hungary, Slovakia, the Czech Republic and Croatia.

The 31 individuals of *F. hepatica* investigated could be grouped into 3 different clusters for NAD1 and in 2 different clusters for COX1. Differences were more pronounced in the *nad1* gene than in the *cox1* gene.

Our data indicate that the Austrian *F. magna* population is rather homogenous and might even be the result of a single introduction into Austria, while the Austrian *F. hepatica* population is, as expected and as also known from other countries, rather diverse.

The antigen profile reveals 3 proteins which are shared in common. Many other antigens were used by *F. hepatica* or *F. magna* and are possible species specific identification characteristics which can be used in diagnosis.

Further investigations are needed to characterize the antigens and to identify cross-reactions between these two species.

Parasites of white-eye bream *Ballerus sapa* (Pallas, 1814), perch *Perca fluviatilis* L, 1758 and other fish species from the Austrian Danube

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This work presents the results of the parasitological investigation of fish from the Danube River which were sampled between July and September 2014 from four locations. The fish were collected by the use of gill nets and electrofishing. The sites are running from west to east: Engelhartzell (Jochenstein), Voest Hafen Linz, Enghagen and Kuchelauer Hafen at Vienna. In the course of the study 93 specimen from 6 different fish species were caught (sampling size noted in brackets), including perch *Perca fluviatilis* (35) white-eye bream *Ballerus sapa* (29), bleak *Alburnus alburnus* (13), perch-pike *Sander lucioperca* (6), roach *Rutilus rutilus* (5) and chub *Squalius cephalus* (5). In total 13 parasite-taxa could be detected: Two monogeneans: *Ancyrocephalus paradoxus* and *Paradiplozoon sapae* and the ciliate *Ichthyophthirius multifiliis* on the gills. From the skin *Trichodinella* sp. (Ciliophora) and *Gyrodactylus* sp. (monogenea) were recovered. *Tracheliastes maculatus*, a crustacean parasite was also found on the skin. Metacercariae of the digenean trematode *Posthodiplostomum cuticola* could be detected on the skin and the fins. In the digestive tract two Acanthocephala, namely *Acanthocephalus lucii* and *Pomphorhynchus laevis* were detected. *Camallanus truncatus*, a nematode, could be found in the stomach of *S. lucioperca*. *Caryophyllaeus laticeps*, a monozoic cestode, was found in the intestine of *B. sapa*. In the eyes of fish the metacercariae of the digeneans *Diplostomum* sp. and *Tylodelphys* sp. were also detected.

In general, it should be mentioned that all fish except *A. alburnus* from the Kuchelauer Hafen were in a very good state of nutrition and Fulton's condition factor was above average. The values of prevalence and intensity of parasitic infection have to be classified as rather low. With a few exceptions, such as *P. cuticola* and *P. laevis*, which could be detected at all study sites, most parasite-taxa were represented by only few individuals.

The two parasite species *A. paradoxus* and *T. maculatus* however have rarely been described from the Austrian Danube before.

***Balamuthia mandrillaris* infections**

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Balamuthia mandrillaris is a free living amoeba (FLA), occurring worldwide in soil and freshwater habitats. The organisms occurs as motile (feeding, multiplying and cytopathic) trophozoite, and as cyst, a dormant form capable of resisting adverse environmental conditions. *B. mandrillaris* is also the causative agent rare but highly lethal *Balamuthia* amoebic encephalitis (BAE) in humans (>200 reported cases) and other mammals. Initially, most reports on BAE concerned AIDS- or otherwise severely immunocompromised patients, and *B. mandrillaris* was considered to be a classical opportunist similar to *Acanthamoeba* spp. Later, the disease was also found in children and adults without evidence of an underlying acquired or innate immunodeficiency. Nevertheless, some immunological deficit – possibly only transient – as it might result from other infections or debilitating chronic diseases and habits, cannot be ruled out, and may be a prerequisite for developing BAE. This concept is supported by experimental infections in a mouse model (Kiderlen et al. 2015). Due to the low case numbers and the long time between presumed infection and onset of symptoms (weeks to many months), the mode of infection is not completely clear. Aspiration of dust contaminated with cysts or primary infections of the skin may be followed by hematological spreading with predilection for the central nervous system. In animal experiments a nasal route along the olfactory nerve and through the cribriform plate has also been demonstrated (Kiderlen & Laube 2004). Recently, BAE has gained public attention due to three clusters of patients that had received solid organ transfer from donors whose *B. mandrillaris* infection had escaped notice (Kaul et al. 2014).

The first case of *Balamuthia* amoebic encephalitis occurred in the USA in a pregnant mandrill baboon (Visvesvara 1990). In Europe, the first case was reported in Czech child 1998 (Kodet R et al. 1998), and in Germany the first case was recorded 2011 in a female gorilla (Mätz-Rensing et al. 2011).

Our institution performs research on the biology of *B. mandrillaris* infections and provides assistance regarding FLA diagnostics.

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Aktuelle Weltseuchenlage

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Octenidine dihydrochloride killing *Trichomonas vaginalis*

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Trichomonas vaginalis is the causative agent of the most common non-viral sexually transmitted disease. The infection is associated with a wide spectrum of complications like low birth weight, infertility and increased susceptibility to HIV. Rising numbers of reports on metronidazole-resistant strains drive the search for new compounds. In the present study, the *in vitro* effects of the common antiseptic octenidine dihydrochloride against *T. vaginalis* were tested on metronidazole-resistant and -susceptible strains. The experiments were performed under microaerophilic conditions in three different media varying in their protein concentrations. It was shown that octenidine dihydrochloride has a strong effect on metronidazole-resistant as well as -susceptible strains. The EC₉₅ values range from 1.81 µg/ml to 98.44 µg/ml for the metronidazole-susceptible and from 1.64 µg/ml to 101.19 µg/ml for the metronidazole-resistant strain, depending on incubation time and protein concentration. The pharmaceutical product approved for the treatment of vulvovaginal candidosis as well as bacterial infections contains 1000 µg/ml octenidine dihydrochloride and thus seems to be an effective alternative for the treatment of trichomonosis, particularly in mixed vaginal infections or cases involving metronidazole-resistant strains.

Supported by Medical University Vienna and Schülke & Mayr GmbH

Opportunisten

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Larvae causing nasopharyngeal myiasis of red deer (*Cervus elaphus*) from Vorarlberg and Tyrol – morphological and molecular assessment

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Nasopharyngeal myiasis are caused by larvae of bot flies (Diptera, Oestridae), which have evolved a high specificity for their hosts. In 2014 bot flies (n= 916) were collected from 137 (57.8%) out of 237 red deer (*Cervus elaphus* Linnaeus, 1758) hunted in Vorarlberg and Tyrol (Austria). After being stored in 70% ethanol larvae were identified to species level and developmental stage using morphological and morphometric keys (e.g. measurements of body length and width). Larvae were also molecularly characterized by PCR amplification and partial sequencing of the mitochondrial cytochrome oxidase 1 gene. Morphological and genetic data were concordant in identifying larvae as belonging to *Cephenemyia auribarbis* and *Pharyngomyia picta*. Genetic variations were also found within the specimens collected in both geographical locations.

***Giardia lamblia* thioredoxin reductase: a well-known enzyme with unusual characteristics**

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Like most organisms, the microaerophilic protist parasite *Giardia lamblia* expresses thioredoxin reductase (TR), an important factor of the cellular redox system. The main role of TR is the reduction of thioredoxin (Trx), an electron carrier protein that reduces/activates a wide range of substrates. One of Trx's most important substrates is peroxiredoxin which removes hydrogen peroxide and, thereby, protects the cell from oxidative damage. Interestingly, TR does not only reduce Trx but also other substrates, such as molecular oxygen or compounds with a nitro group. Amongst the latter is the nitroimidazole drug metronidazole which constitutes the gold standard in the treatment of pathogens with an anaerobic/microaerophilic metabolism. As metronidazole has to be reduced at its nitro group in order to be rendered toxic, TR might be an important factor in metronidazole-mediated toxicity.

It was our goal to further elucidate the role of TR in the redox system of *G. lamblia* and its importance as a metronidazole reducing factor. To this end a., the *Giardia* proteome was searched for factors interacting with TrxR, and b., expression of TR was manipulated genetically in order to assess the importance of this enzyme for metronidazole reduction. Surprisingly, no thioredoxins could be identified as interacting proteins of TR and also all thioredoxin-like proteins tested were not substrates of TR. In immunolocalization experiments, TR was found to localize to the median body, a structure assumed to act as a storage depot in the *Giardia* cell, and not in the cytoplasm as seen in most organisms. Over-expression of TR, driven from an introduced plasmid, rendered *G. lamblia* more susceptible to metronidazole and other nitro drugs but not to the extent expected. Taken together, our data imply that the role of TR in *G. lamblia* could be fundamentally different from the role it has in other organisms.

Epidemiology of Cystic Echinococcosis in the Province of Moyen-Ogooué, Gabon, Central Africa

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Aim: Cystic echinococcosis (CE) is thought to have an almost worldwide distribution. CE is listed as occurring in most regions of sub-Saharan Africa with varying prevalence, but epidemiologic data for Central Africa are to date virtually non-existing. We therefore conducted an epidemiologic field survey in central Gabon – a rural Central African tropical ecosystem.

Methods: Volunteers were informed about the project and after informed consent was given, abdominal ultrasound examination was performed and blood was drawn for serologic analyses. A questionnaire on risk factors was completed. For serologic analysis, a commercial indirect haemagglutination assay was used (Celloghost Echinococcosis, Siemens) as screening test and if positive, a Western Blot was performed (Echinococcus WB, LDBIO Diagnostics) as confirmatory testing. In addition, canine stool samples were collected for microscopic and PCR-analysis.

Results: In total, 347 patients were screened by ultrasound and serologic analyses for cystic echinococcosis. 198 (57.1%) were female and 149 (42.9%) were male. Patients from 19 different communities in the province of Moyen-Ogooué were included. No cystic lesions compatible with cystic echinococcosis were detected in ultrasound screening. Nine volunteers (2.6%) were positive for echinococcosis in the IHA screening test. None was positive in the Western Blot. 46 canine stool samples were analysed microscopically and via PCR. None of the samples was positive for echinococcosis.

Conclusion: This epidemiologic pilot study provides preliminary epidemiologic data for CE in a Central African ecosystem. No suspected case of CE could be identified and none of the collected canine stool samples contained DNA of *Echinococcus granulosus*. Although sample size was limited and further collection of canine stool samples is currently performed, we conclude that there is currently no evidence for autochthonous transmission of CE in this Central African tropical ecosystem.

Intra-cystic drug concentration of albendazole and its active metabolite albendazole-sulphoxide in human cystic echinococcosis: a systematic review and analysis of individual patient data

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Aim: Albendazole - the first-line anthelmintic drug for medical treatment of cystic echinococcosis - is metabolized *in vivo* to the active derivative albendazole-sulphoxide (ABZ-SO). Target site ABZ-SO concentrations in the echinococcal cyst mediate the anthelmintic effect in human cystic echinococcosis. We present a systematic review of current evidence on intra-cystic target site concentrations of ABZ-SO.

Methods: A systematic review and pooled analysis of individual patient data was performed from all available published reports. The main outcome of this systematic review was the description of target site concentrations of ABZ-SO and potential co-variables.

Results: Pharmacokinetic data of 121 individual cysts were analysed. There was no correlation between plasma (median 245µg/L; 25th-75th percentile: 132 - 518µg/L) and cyst fluid (median 200µg/L; 25th-75th percentile: 94 - 434µg/L) ABZ-SO concentrations ($\rho = -0.03$, $p = 0.76$). Intra-cystic drug concentrations were not associated with sex, and treatment duration. The use of praziquantel in combination with ABZ was associated with higher ABZ-SO concentrations in plasma (540µg/L (25th – 75th percentile: 255 - 1020µg/L) vs. 240µg/L (25th – 75th percentile: 132 – 493µg/L); $p = 0.04$) but not in cysts (220µg/L (25th – 75th percentile: 170 - 510µg/L) vs. 199µg/L (25th – 75th percentile: 92 – 425µg/L), $p = 0.36$). Relative drug concentrations in hepatic cysts were higher than in other cysts (0.8 (25th – 75th percentile: 0.4 – 2.4) vs. 0.4 (25th – 75th percentile: 0.2 – 0.8); $p = 0.05$). Target site concentrations were higher in calcified than non-calcified cysts (median 897µg/L; 25th to 75th percentile: 504 - 2763µg/L compared to 245µg/L; 25th – 75th percentile: 143 - 499µg/L; $p = 0.03$). There was a trend towards higher concentrations in smaller sized cysts ($\beta = -17.2\mu\text{g/L per cm}$, 95th CI: -35.9 – 1.6; $p = 0.07$).

Conclusion: This systematic review demonstrates that mean intra-cystic drug concentrations are similar to plasma concentrations on a population level. However, in individual patients plasma concentrations are not directly predictive for cyst concentrations. The use of booster drugs was not associated with higher intra-cystic ABZ-SO concentrations in this review.

Malaria vaccine

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Acanthamoeba* spp- as possible host organisms for the pathogen *Burkholderia pseudomallei

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Burkholderia pseudomallei is a gram negative bacterium and the causative agent of melioidosis which is associated with different symptoms including severe pneumonia and septicemia. Moreover, melioidosis can progress seriously in individuals with an altered immune response and underlying diseases such as diabetes. Melioidosis is acquired by inoculation of the pathogen through skin lesions, inhalation by bacteria-containing aerosols and also by ingestion. Until recently, the disease was considered to occur mainly in Northeast Australia and Southeast Asia but in the past years, more and more cases have been reported. *Acanthamoeba* spp. are free-living amoebae found in soil, water, dust and even in air samples and as they produce extremely resistant cysts they are also known to be important reservoirs for numerous bacterial pathogens, e.g. *Legionella pneumophila*.

The aim of this project was to evaluate the diversity of *Acanthamoeba* spp. in soil samples from African regions potentially endemic for melioidosis and, together with our partner-lab, prove the hypothesis that free-living amoebae function as host cells for survival and multiplication of *Burkholderia pseudomallei* in the environment.

During the current study soil samples were collected in Burkina Faso, Ethiopia and Madagascar and screened for amoebae and *B. pseudomallei* in parallel. Pure cultures of *Acanthamoeba* spp. were obtained on non-nutrient agar plates and all isolated amoebae were genotyped by DNA sequencing.

As yet out of 36 soil samples all were positive for more than one species of *Acanthamoeba* exhibiting in total six various genotypes out of all three different morphological groups.

Supported by ERAfrica, OEAD

Evidence for stable endemic sandfly populations in the light of migration streams into Austria

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Sandflies (Diptera: Psychodidae: Phlebotominae) are the vectors of medico-veterinary pathogens, including *Leishmania* spp., *Bartonella* spp. and Phleboviruses. In Central Europe, leishmaniasis is a rare disease diagnosed almost exclusively in travellers, soldiers or migrants coming from tropical or subtropical countries. Particularly, in the Eastern Mediterranean Region and the Middle East, leishmaniasis are a major public health problem fortified through the act of war resulting in a massive stream of refugees into Europe.

During an entomological study in July and August 2012 and 2013, sandfly trapping was performed at two capture sites in Eastern Austria where sandflies had been detected. The found specimens were identified as *Phlebotomus (Transphlebotomus) mascittii* Grassi 1908. In several south-eastern regions of Austria sandfly populations were shown to be stable if not increasing. Furthermore, sandfly trapping performed in southern Styria in July 2015, underpinned this statement. A possible circulation of *Leishmania* spp. and Phleboviruses might become an important issue.

Efficacy of Biotrue™ multi-purpose solution against *Acanthamoeba* strains

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Acanthamoeba keratitis is a progressive sight-threatening corneal infection caused by *Acanthamoeba*. The use of ineffective contact lens disinfecting solutions is one of the most important risk factors for this infection. Thus, contact lens wearers are most at risk. This study concerns a recently marketed multi-purpose contact lens solution, Biotrue™, tested for its efficacy against *Acanthamoeba* trophozoites and cysts by using the most probable number technique for amoebic enumeration. *Acanthamoeba castellanii* ATCC 50373 and an environmental strain of *Acanthamoeba* genotype T4 isolated from tap water in Istanbul were used during the experiments. Biotrue™ achieved total kill (more than a 3-log reduction) of trophozoites of both strains before the manufacturer-recommended disinfection time (4 h). In contrast, this solution had limited cysticidal activity against the ATCC strain but more against the environmental strain, with log reductions of 0.47 and 1.81, respectively, after 4 h of exposure. These data suggest that this multi-purpose solution is more effective at killing trophozoites than cysts. Further studies among other *Acanthamoeba* strains are needed to determine the efficacy of this solution.

Comparative Nematode Glycomics

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Glycans cover the surfaces of all cells and are frequently components of secreted proteins, whether of host or parasite origin. Of the various types of glycan, we focus on those covalently linked to asparagine residues of proteins (N-glycans) and have adopted an analytical workflow with HPLC fractionation followed by MALDI-TOF mass spectrometry in combination with enzymatic or chemical treatments and MS/MS fragmentation. In terms of nematodes, we have been analysing the N-glycans of the model worm *Caenorhabditis elegans* for some fifteen years and now, by analysing the glycans of mutant worms and supplementing the data with NMR, show the presence of an unusual bisecting galactose residue which can be fucosylated. In total, *C. elegans* can modify its N-glycans with four fucose residues; however, a maximum of three fucose residues is observed for the necromenic worm *Pristionchus pacificus* and for the parasites *Oesophagostomum dentatum* and *Haemonchus contortus*; in the case of *Trichuris suis* up to two fucose residues were detected. Thus it appears that the non-parasitic nematode has the most complex N-glycome, whereas the parasitic worms are simpler. Nevertheless, parasite glycomes are quite different as compared to their mammalian hosts and the exact knowledge of glycan structures has repercussions for production of recombinant glycoprotein vaccines and for an understanding of the interaction of parasites with host immune systems.

Yan et al (2015) *Electrophoresis* 36, 1314; Yan et al (2015) *Mol Cell Proteomics* 14, 2111; Paschinger and Wilson (2015) *Glycobiology* 25, 585.

KP is an FWF fellow (grants P21946 and P25058 from the Austrian Science Fund); this work is also supported by grant P23922 to IBHW.

Fascioliasis (liver fluke disease) in Styrian dairy farms - prevalence and success of control measures

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Fasciola hepatica infestations are widespread in Austrian cattle and sheep herds and occur predominantly in humid and rainy regions. Animals suffering from fascioliasis (liver fluke disease) may develop chronic diarrhoea and show an increased incidence of metabolic disorders. Financial losses for dairy farms are mainly caused by milk quality problems and by a reduced milk production up to 10 - 15%. The economic damage to fattening farms mainly results from a reduced growth performance (up to 10%) as well as from an app. 4 - 6 weeks prolonged fattening period.

People can develop a fascioliasis after eating unwashed plants or windfalls, sporadically also by consuming uncooked infested livers of cattle and sheep. Due to the compliance with hygienic measures and the post-mortem inspection of all cattle at slaughter, the incidence of this disease in humans in Austria is very low. Only one or two human cases per year have been registered during the last 15 years.

In order to get an overview of the prevalence of liver fluke disease in Styrian dairy farms and to offer the farmers a basis for a required improvement of grazing management or a specific veterinary treatment, the Department of Veterinary Administration of the Styrian Government together with the Styrian Animal Health Service (AHS) implemented a monitoring program based on tank milk serology.

In January 2014 the serological testing of bulk milk from a total of 4,912 dairy farms was carried out by the veterinary laboratory of the Styrian Department of Veterinary Administration. According to this survey 15.5% of the dairy farms showed a positive and 29.5% a low positive result in terms of the *Fasciola hepatica* ELISA. Comparing the test results from different districts, the highest number of positive results could be detected in the districts of Murau (38.5% and 42.2% respectively) and Liezen (26.7% and 46.5%).

So as to determine, if appropriate treatment measures by contracted veterinarians in member farms of the AHS provided an improvement, the study was repeated in January 2015. In the total of 4,706 tested farms, there was a reduction in the proportion of positive evidence by 2.9%, of the low positive evidence by 4.6%. Comparing the results broken down by AHS membership, a significant reduction of 4.0% could be found in the 3,437 AHS-farms both in positive and low positive tank milk samples. In non-AHS-farms however no significant differences between the study passages were detected.

The results show that the prevalence of liver fluke disease in cattle herds can be lowered by high quality veterinary advice and targeted control measures in the context of the Animal Health Service. This also helps to reduce the risk of human fascioliasis.

HIV-Therapie

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Tuberculosis – a new threat in Europe

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Detection of microbial pathogens in ticks collected in Austria

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Ticks are the second most common hematophagous vectors after mosquitos and can transmit a huge number of various pathogens that can lead to human disease. Cases of tick-borne diseases are often underestimated due to a lack of specific symptoms in the patients. Therefore patients who have been bitten by an infected tick might be suffering from a tick-borne disease which could be mistaken for another illness.

Aim of this study was to evaluate the prevalence of microbial pathogens in ticks collected in Austria with the PCR/reverse line blot hybridization technique.

A total number of 554 *Ixodes ricinus* ticks have been screened by using this method. The pathogen with the highest prevalence detected was *Borrelia burgdorferi* sensu lato in 25.6% with *B.afzelii* (56.3%) being the most frequently detected species within these positive ticks. followed by *B.burgdorferi* sensu stricto (26.8%) and *B.valaisiana* (25.4%). *B.garinii/B.bavariensis*, *B.lusitaniae* and *B.spielmanii* have been detected in 19.7%, 3.5% and 0.7% of the *Borrelia* positive ticks, respectively. Co-infections with multiple *Borrelia* strains occurred in 27.4% of the positive ticks. *Rickettsiae* spp. represented the pathogen group with the second highest prevalence in 16.8% of the ticks. The species detected were *R.helveticum* (41.9%), *R.monacensis*(2.2%), *R.slovaca* (1.1%) and interestingly *R.raoultii* in a very high number (40.9% of the *Rickettsia* positive ticks) with a hotspot in Vienna (Lainzer Tiergarten). This finding still needs to be further investigated due to the fact that *R.raoultii* has not been detected in *I.ricinus* ticks before.

Candidatus Neoehrlichia mikurensis, *Babesia* spp. (*B.venatorum*, *B.divergens*, *B.microti*) and *Anaplasma phagocytophilum* have been detected in 4.3%, 2.7% and 0.7%, respectively. No *Coxiella burnetti* positive ticks were found.

International Health Regulations: Was ist neu?

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Internationale Gesundheitsvorschriften sind gesetzlich bindende Vorschriften für 196 Länder der Welt zur Krankheitsprävention und -kontrolle mittels Public Health Maßnahmen. Ziel dieser Vorschriften sind eine verbesserte Detektion und ein geregeltes Meldewesen von potentiell international relevanten Gesundheitsgefahren/-notfällen. Die WHO spielt hierfür eine zentrale Rolle in der Koordination.

Im Vortrag werden insbesondere die aktuellen Vorschriften zur Gelbfieberimpfung und Polioimpfung im internationalen Reiseverkehr besprochen. Außerdem wird erörtert unter welchen Umständen eine Meningokokkenimpfung vorgeschrieben ist.

Acanthamoeba encephalitis

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Besides their importance as causative agents of an often seriously progressing keratitis occurring predominantly in contact lens wearers *acanthamoebae* can also cause granulomatous amoebic encephalitis (GAE). This disease occurs almost exclusively in patients who have impaired defence mechanisms or who are chronically ill. Organ transplantation, pregnancy, alcoholism, liver disease, haematological disorders, renal failure, diabetes mellitus, splenectomy, tuberculosis, steroid treatment, chemotherapy and AIDS have been described as predisposing factors. GAE has an insidious onset and is a subacute or chronically progressing disease with an incubation period of several weeks or even months. The mode of infection is mostly aerogenic or by contact with contaminated water, the lower respiratory tract or skin lesions functioning as portals of entry. In many cases inflammation can already be observed at these primary foci. The amoebae finally invade the brain and also other organs by haematogenous or rather perivascular dissemination. Typical symptoms are severe headache, confusion, dizziness, drowsiness, nausea, seizures, aphasia, anorexia, and somnolence. High fever, hemiparesis and coma may be the consequence. Usually, multiple necrotizing lesions and diffuse oedemas are found, the cerebral hemispheres being the most affected, but also the cerebellum and the brainstem can be involved. GAE is an extremely rare but often fatal infection due to the unavailability of efficiently effective treatment.

The first clearly identified human case of *Acanthamoeba* GAE occurred in the USA in a patient with Hodgkin's disease (Jager & Stamm 1972) and the first case of GAE in an AIDS-patient was recorded in 1986, also in the USA (Wiley et al. 1987). In Europe, the first case was reported in an AIDS patient in Italy in 1992 (DiGregorio et al. 1992), the first recorded Austrian case of GAE occurred in 2004 (Aichelburg et al. 2008).

Our institution is the Austrian reference laboratory for *Acanthamoeba* diagnostics, during the past 20 years 3 cases of GAE were diagnosed.

Studying Golgi biogenesis using *Trypanosoma brucei*

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The Golgi lies at the centre of the secretory pathway, receiving newly-synthesized proteins from the endoplasmic reticulum, processing them through modifications to the bound oligosaccharides, and then sorting them to their appropriate destinations. As with other cellular organelles, the Golgi undergoes duplication during the cell cycle and partitioning during mitosis, so as to ensure propagation through successive generations. The process of duplication - making another copy of the Golgi - has been difficult to study since most cells have many, often hundreds, of Golgi, making it difficult to follow the appearance of new ones. We have solved this problem by focusing on protozoan parasites, some of which have only one Golgi that can be followed using GFP technology. Through studying the Golgi in *Trypanosoma brucei* (the causative agent of sleeping sickness), we have been able to tackle the mechanism that ensures duplication and partitioning of this organelle. Our results, using photoactivatable GFP in living and permeabilised cells, point to a key role for the old Golgi in constructing the new.

Impfungen beim immunsupprimierten Reisenden

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Molecular evidence of bacterial and protozoal pathogens in ticks collected from dogs from eastern Austria

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During a previous study ticks have been collected from dogs that were walked daily in a region in the east of Austria, which took place in a time period of 11 months (February-December) in 2008. From this tick collection 174 ticks were selected at random, including 75, *Dermacentor reticulatus* (47 female, 28 male), 44 *Haemaphysalis concinna* (21 females, 6 males and 16 nymphs) and 55 *Ixodes ricinus* (52 females, 3 males) ticks.

To screen the ticks, the Reverse Line Blot (RLB) hybridization technique has been used, which is a technique that screens multiple ticks for the presence of DNA of multiple pathogens at once. Briefly, the RLB consists of a negatively charged nitrocellulose membrane that has up to 43 (geno)species specific oligonucleotides covalently bound to it with the use of an amino-linker attached to the 5' side of the oligonucleotide probe and through the use of a miniblotted. To screen for pathogen DNA, whole tick DNA extractions are subjected to several genus specific PCRs with biotin-labeled reverse primers. The resulting PCR products are then loaded perpendicularly to the bound oligonucleotide probes and detection takes place through the use of chemiluminescence resulting from a substrate for the horseradish peroxidase-streptavidin conjugate that has been hybridized to the biotin attached to the PCR product. For the genus specific PCRs the following targets were used: For *Anaplasma/Ehrlichia* spp., the 16s rRNA gene, for *Babesia/Theileria* spp. the 18s rRNA gene, for *Rickettsia* spp. the 23-5s intergenic spacer and for *Borrelia* the 5-23s intergenic spacer.

In these ticks the DNA of the following pathogens was detected: *Anaplasma phagocytophilum*; *Borrelia afzelii*; *B. burgdorferi* sensu stricto; *B. garinii*; *B. lusitaniae*; *B. spielmanii*; *B. valaisiana*; *Candidatus Neoehrlichia mikurensis*; *Rickettsia helvetica*; *R. raoultii*; *Theileria (Babesia) microti*; Genus specific catch-all only signals have been sequenced and new probes have been designed.

Kranke Reiseheimkehrer: Eine diagnostische Herausforderung

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Is classification of Leprosy as an NTD a game changer?

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[1] The struggle for a world free of leprosy is a success story with many chapters. Once a very vertical program, it is the ambition of *Austrian Leprosy Relief Association (ALRA)* and its partners united under the umbrella of the *International Federation for a World free of Leprosy (ILEP)* to contribute making the successes of anti-leprosy work instrumental in strengthening primary health care (PHC) as defined in Alma Ata 1978.

[2] In many countries (especially in Africa South of the Sahara, SSA), the struggle to overcome Leprosy and Tuberculosis (TB) is united in one country program. This is not only due to the biological similarities of the two mycobacteriae, and history of medicine. In fact, it is closely related to the development of European fundraising techniques that have proven unintended negative impact on overcoming leprosy.

[3] Increasingly, and following the WHO's classification, leprosy is regarded as one of the Neglected Tropical Diseases (NTDs). This creates long awaited possibilities to finally bringing back anti-leprosy work to actually strengthening and no longer weakening primary health care. For sure, the present focus on NTDs (that are part of the Sustainable Development Goals, SDGs) will have significant impact on policy levels. Again, impact is likely to be both intended positive (increased coordination, synergies) and negative (efficiency losses due to political competition within health ministries, and danger of NTD becoming yet another vertical program).

[4] From ALRA's perspective, three initiatives are especially worth noting:

- <http://www.leprosy-information.org/resource/ten-steps-guide-health-promotion-and-empowerment-people-affected-neglected-tropical>
- <http://www.who.int/features/factfiles/wash-ntds/en/>
- Toolbox development led by Netherland Leprosy Relief (NLR)

These are encouraging new developments that closely related to the nature of the SDGs replacing the Millennium Development Goals (MDGs).

ILEP's strategy for achieving a world free of leprosy <http://www.ilepfederation.org/wp-content/uploads/2015/07/ILEP-strategy-for-2015-2018-.pdf> that sits on three pillars (stop transmission, prevent disability, achieving inclusion) fits well into the framework of the SDG. With ILEP being part of the NGDO network, anti-leprosy work is entering an exciting new phase. It can become a game changer both in regards to fundraising, and in advancing the accelerated efforts to overcome the NTDs.

DNA-Barcoding of the Austrian mosquito species (Diptera: Culicidae) inventory

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Mosquitoes are important vectors for a variety of pathogens including flaviviruses (West Nile Virus, Dengue Fever and Yellow Fever), nematodes (filarioid helminths) and protozoa (*Plasmodium* spp.). To understand vector–pathogen dynamics in Austria, a better knowledge of mosquito species ecology as well as detailed information on seasonal and spatial distribution patterns of mosquito communities is essential. Therefore a comprehensive mosquito species inventory for Austria is crucial. At present, several taxonomically challenging cryptic taxa seem to have been neglected and disregarded, particularly morphologically highly similar sibling species or members of species-complexes. Molecular methods such as DNA barcoding might enhance taxonomic resolution and thus help to establish a valid species inventory of Austrian mosquitoes.

Mosquitoes were monitored from April to October in 2014 and 2015 at 29 permanent and 30 non-permanent sampling sites, distributed across Eastern Austria (Vienna, Burgenland and Lower Austria). Female mosquitoes were collected twice a month for 24 hours, using BG-Sentinel traps equipped with carbon dioxide as an attractant. Within these sampling periods more than 29,000 female mosquitoes comprising 26 species were sampled. Additionally mosquito samples collected by collaborating institutions were included into the study in order to ensure that at least four individuals of each morphologically identified species were sequenced to obtain partial mitochondrial *cytochrome oxidase I* genetic sequences.

In this study we aim to update the mosquito species inventory of Eastern Austria in order to facilitate risk assessment for mosquito-borne diseases in Austria. We further discuss adequacy of DNA-barcoding as tool to improve and support the identification of morphologically cryptic mosquito species.

This research was funded by the ERA-Net BiodivERsA, with the national funders FWF I-1437, ANR-13-EBID-0007-01 and DFG BiodivERsA KL 2087/6-1 as part of the 2012-13 BiodivERsA call for research proposals.

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