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Société Suisse de Médecine Tropicale et de Parasitologie
Schweizerische Gesellschaft für Tropenmedizin und Parasitologie
Società Svizzera di Medicina Tropicale e di Parassitologia
Swiss Society of Tropical Medicine and Parasitology

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Disease Ecology in a Changing World

Annual meeting of the
Swiss Society of Tropical Medicine and Parasitology (SSTMP)

Jongny-sur-Vevey, 23-25 October 2008

This annual meeting is sponsored by:

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Disease Ecology in a Changing World

Programme of the annual meeting of the Swiss Society of Tropical Medicine and Parasitology (SSTMP), Jongny-sur-Vevey, 23-25 October 2008

Thursday 23rd October

Time	Title of presentation	Speaker
	12.00 Lunch (optional, no booking required)	
14.00-14.15	**Welcome**	**C. Lengeler**
14.15-15.00	Keynote 1: **Vectors of emerging diseases in central Europe and their control**	**F. Schaffner,** IPZ
15.00-15.45	Keynote 2: **Emerging communicable diseases: threats and responses in Switzerland**	**T. Eckert,** BAG
	Tea break	
Scientific session 1: **Clinical studies**	*Chair: F. Chappuis*	
16.15-16.30	1a: **Screening blood donors for malaria infections**	I. Felger
16.30-16.45	1b: **Pharmacogenetics and pharmacokinetics in malaria patients**	E.M. Hodel
16.45-17.00	1c: **Chagas disease among latin-american immigrants in Geneva, Switzerland: preliminary results of a seroprevalence study**	Y. Jackson
17.00-17.15	1d: **IMPAMEL III - safety and efficacy of the 10-day melarsoprol schedule against late stage *T.b. rhodesiense* sleeping sickness**	I. Küpfer
17.15-17.30	1e: **Three cases on Intestinal Capillariasis in Lao People's Democratic Republic**	P. Soukha-thammavong
17.30-17.45	1f: **Selection and application of synthetic peptides for serodiagnosis of helminth infections**	C. List
	19.00 Dinner (optional but booking required)	

Friday 24th October

Time	Title of presentation	Speaker
08.30-09.15	Keynote 3: **Urban echinococcosis**	**P. Deplazes,** IPZ
09.15-10.00	Keynote 4: **Mapping vector-borne disease risks and movements on a global scale**	**A. Tatem,** Univ. Oxford
	Tea break	
Scientific session 2: **Public health**	*Chair: H.P. Marti*	
10.30-11.00	Keynote 5: **The discovery of the AADs, a new class of anthelmintic compounds**	**R. Kaminsky,** Novartis
11.00-11.15	2a: **Swiss support to the national programme for insecticide-treated nets in Tanzania (NETCELL)**	C. Lengeler
11.15-11.30	2b: **Innovations in improving access to malaria treatment in Tanzania: the ACCESS Programme**	C. Lengeler
11.30-11.45	2c: **Patient costs during the intensive and continuation phases of tuberculosis treatment in Central Asia**	R. Ayé
11.45-12.00	2d: **Water recontamination at point-of-consumption – improved supply sources and home-based water treatment will not guarantee safe drinking water**	S. Rufener
12.00-12.15	2e: **Emergence of the eye worm *Thelazia callipaeda* in southern Switzerland and occurrence of its potential vector *Phortica* spp.**	M. Schnyder
	Lunch	

Time	Title of presentation	Speaker
Scientific session 3 (parallel session)***:*** **Parasitology** *Chair: H.-P. Beck*		
14.00-14.15	3a: ***Echinococcus multilocularis*** **phosphoglucose isomerase (EmPGI): a moonlighting enzyme?**	B. Stadelmann
14.15-14.30	3b: **How *Giardia lamblia* deals with stress**	C. Spycher
14.30-14.45	3c: **Transcriptional profiling of differentiation in *Giardia lamblia***	L. Morf
14.45-15.00	3d: **Neogenesis and maturation of a naturally pulsed Golgi-like organelle system during stage-differentiation in *Giardia lamblia***	C. Konrad
15.00-15.15	3e: **Essential role of P-gp in *Toxoplasma gondii* replication**	I. Bottova
15.15-15.30	3f: ***Toxoplasma gondii*** **P-glycoprotein: insights into essential cellular functions**	S. Sonda
15.30-15.45	3g: ***Neospora caninum*** **and the interaction with murine bone marrow-derived dendritic cells**	M. Strohbusch
Scientific session 4 (parallel session)***:*** **Clinical case discussions** *Chairs:* M. Bouvier Gallacchi and F. Chappuis		
14.00-15.45	Surprises	
Tea break		
16.15-16.45	**Invited presentation 1: The new Global Malaria Action Plan**	T. Teuscher
16.45-17.05	**Invited presentation 2: Comparative genomics of membrane proteins in tropical parasites and their hosts**	N. Fankhauser
17.15-18.15	**General Assembly of Swiss Society of Tropical Medicine and Parasitology** (see separate agenda)	
19.30 Gala Dinner with SSTMP award (separate booking and payment required)		

Saturday 25th October

Time	Title of presentation	Speaker
09.00-09.45	Keynote 6**: Emerging threats of animal diseases with a focus on Blue Tongue Disease**	**L. Perler,** BVET
09.45-10.30	Keynote 7**: The predicted health consequences of global climate change**	**F. Matthies,** WHO Europe Office
	Tea break	
	Scientific session 5: **Veterinary diseases** *Chair:* A. Mathis	
11.00-11.15	5a: **Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by in vitro cultivation and PCR**	C.F. Frey
11.15-11.30	5b: **Validation of a Western Blot for the detection of anti-*Trichinella* spp. antibodies in domestic pigs**	C.F. Frey
11.30-11.45	5c: **Distribution and abundance of biting midges, the potential vectors of bluetongue disease in Switzerland**	C. Kaufmann
11.45-12.00	5d: **Flight performance of the Asian tiger mosquito *Aedes albopictus* (Diptera: Culicidae)**	C. Kaufmann
12.00-12.15	5e: **Ticks and tick-borne pathogens from wildlife in the Free State province, South Africa**	M. Berggoetz
12.15-12.30	**Closure**	**C. Lengeler**
	Lunch (optional, no booking required)	

Posters

Number	Title of poster	First author
P1	***Echinococcus multilocularis* vesicles impair co-stimulatory molecule expression on human myeloid dentritic cells and their T cell stimulatory effect**	M. Margos
P2	**Monkey malaria in a European traveller returning from Malaysia**	A. Kantele
P3	**Arbovirus transmitted by mosquitoes in southern Switzerland: a potential risk?**	T. Yang

Disease Ecology in a Changing World

Abstracts of the annual meeting of the Swiss Society of Tropical Medicine and Parasitology (SSTMP), Jongny sur Vevey, 23-25 October 2008

Scientific session 1: Clinical Studies

Chair: Francois Chappuis

1a

Screening blood donors for malaria infections

Ingrid Felger, Hanspeter Marti, Christoph Hatz

Swiss Tropical Institute, Basel, Switzerland

Transfusion transmitted malaria (TTM) has been described occasionally in Europe and mostly in the US. In Switzerland a case of TTM with fatal outcome was described in 2001 (Frey-Wettstein et al. 2001). Blood banks and Red Cross generally take precautions against transfusion mediated transmission by carefully selecting donors and applying screening tests. The current policy in Switzerland as of April 2007 stipulates permanent deferral of long term residents (> 6 months) in tropical countries unless lab testing was negative. The designated laboratory tests for this purpose are IFA and ELISA. Lab testing is also required for donors returning from endemic areas, who had a fever episode during or after travel. Negative test results will cause a deferral of 6 months, positive results cause deferral for 3 year, after which a further test is needed. This deferral policy leads to a considerable loss of potential blood donors and better tests are warranted to rule out possible transfusion cases.

Our generic Plasmodium qPCR assay targets the 18 S rRNA gene, that is present in the genome of Plasmodia in several copies. Primers and probe were designed from regions of the 18S rRNA gene that are conserved among the four human Plasmodium species, but differ substantially from homologue sequences in humans.

Sensitivity was determined in *P. falciparum* parasite *in vitro* culture serially diluted and in standardized blood samples from an external quality control (Padley et al. 2008). For the other *Plasmodium* species, where no in vitro cultured material is available, positive blood samples confirmed by expert microscopists were used. The detection limit was at a parasitemia of 0.00001% corresponding to 50 parasites/mL whole blood. Thus, the sensitivity of qPCR is 200-fold superior to thick blood film (10 parasites /µL whole blood).

The reliability of travel or residency information from blood donors is poor and should not remain the sole criterion for deferral of blood donations. We aimed for a direct parasite demonstration by PCR because immunological methods cannot discriminate current infection from distant previous travel due to long term antibody titres thus leaving infection status unclear.

Frey-Wettstein M et al. A case of transfusion transmitted malaria in Switzerland Swiss Med Wkly. 2001;131(21):320

Padley DJ and Collaborative Study Group. Establishment of the 1st World Health Organization International Standard for Plasmodium falciparum DNA for nucleic acid amplification technique (NAT)-based assays. Malar J. 2008 Jul 24;7:139.

1b

Pharmacogenetics and pharmacokinetics in malaria patients

Hodel EM[1]*, Zanolari B*[2]*, Mercier* T[2]*, Ley S*[1,a]*, Decosterd LA*[2]*, Olliaro P*[3]*, Genton B*[1]*, Beck HP*[1]

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[2] Clinical Pharmacology Laboratory, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne
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The interaction between the drug, the parasite and the human host determine antimalarial treatment outcome in terms of both efficacy and tolerability. Knowledge of the disposition of the majority of drugs in malaria is generally inadequate. Even less known are genetic differences in drug metabolism, which could variably affect drug exposure and effects.
A better understanding of the disposition and effects of the profiles of genes controlling drug metabolism would allow refining and adapting drug regimens to specific populations.
Therefore, we have developed and validated a single liquid chromatography-tandem mass spectrometry method (LC-MS/MS) requiring 200 µl of plasma for the simultaneous determination of 14 antimalarial drugs and their metabolites which are the basis of the current first-line combination treatments for malaria (Hodel *et al.* in press). The pharmacokinetic profiles generated will be analysed with respect to the individual's pharmacogenetic profile obtained by extension of our DNA chip technology recently developed for malaria drug resistance (Crameri *et al*. 2007). This technique allows the determination of single nucleotide polymorphisms in genes coding for proteins relevant to the metabolism of the main in-use antimalarial drugs. We currently cover 24 SNPs in 9 genes of the cytochrome P450 isoenzyme family and *N*-acetyltransferase 2 genes.

Hodel EM, Zanolari B, Mercier T, Biollaz J, Keiser J, Olliaro P, Genton B and Decosterd LA. A single LC- tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma. *Manuscript submitted*.
Crameri A, Marfurt J, Mugittu K, Maire N, Regos A, Coppee JY et al. Rapid microarray-based method for monitoring of all currently known single-nucleotide polymorphisms associated with parasite resistance to antimalaria drugs. *J.Clin.Microbiol*. 2007;45(11):3685-91.

1c

Chagas disease among latin-american immigrants in Geneva, Switzerland: preliminary results of a seroprevalence study

Yves Jackson, Laurent Getaz, Marylise Holst, Louis Loutan, François Chappuis

Department of Community Medicine and Primary Care, Geneva University Hospitals and University of Geneva, Switzerland

Chagas disease is endemic in Latin America, affecting 8-10 millions persons. Recent migration to non-endemic countries has changed the epidemiology of this disease. An increasing number of cases are reported in Europe. We aimed to evaluate the prevalence of Chagas disease in Latin American immigrants in Geneva.

Latin American immigrants consulting at the Unité Mobile de Soins Communautaires of the HUG are screened with two serological tests (Biokit Chagas™ and Biomérieux ELISA Chagas™). All patients diagnosed with Chagas disease have a complete medical evaluation including screening for heart and digestive tract involvement.

From June to September 2008, 617 persons have been recruited. The majority (84%) of patients are women and the median age is 36 (range: 18-78). The vast majority of patients live in Switzerland without residence permit (undocumented). Countries of origin are Bolivia (n=304), Brazil (n=155), Colombia (n=38), Peru (n=24) and others (n=96). Chagas disease was diagnosed in 80 patients (13%). The prevalence of disease among immigrants from Bolivia is 25.3% (n=78).

Chagas disease is highly prevalent in Latin-American undocumented immigrants living in Geneva. The prevalence among Bolivians is strikingly high, but is consistent with recent serosurveys conducted in Bolivia.

Access of Latin-American immigrants to reliable diagnosis and treatment for Chagas disease should be more widely available in Switzerland. In addition, the risk of local transmission by congenital route, blood transfusion or organ transplant should be tackled.

1d

IMPAMEL III - safety and efficacy of the 10-day melarsoprol schedule against late stage *T.b. rhodesiense* sleeping sickness

Kuepfer Irene[1], Mpairwe Allan[2], Matemba Lucas[3], Schmid Caecilia[1], Burri Christian[1]

[1]Swiss Tropical Institute, Swiss Center for International Health, Switzerland; [2]Lwala Hospital, Kaberamaido District, Uganda, [3]National Institute for Medical Research, Tabora, Tanzania

At the ISCRTC meeting in 2003, the use of a new, abridged 10-day melarsoprol schedule for treatment of second stage *T.b. gambiense* sleeping sickness was recommended. This shorter regimen is socio-economically superior to the standard empirical regimens due to the 50% reduction in hospitalization time and the reduced overall amount of melarsoprol given.
The WHO has recommended the conduct of clinical trials in *T.b. rhodesiense* affected areas. In 2006 the IMPAMEL III program was initiated. The primary objective was to assess the safety, tolerability and efficacy of the 10-day melarsoprol schedule in East Africa.
A non-controlled Phase II proof-of-concept trial including 60 late stage *T.b. rhodesiense* patients was conducted in Uganda and Tanzania. Follow-up visits were scheduled for 3, 6 and 12 months post treatment. In the same study sites and based on the results of the proof-of-concept trial, an additional utilization study with 78 patients was conducted using historic controls as comparisons.
A total of 138 late stage *T.b. rhodesiense* patients were treated with the 10-day melarsoprol schedule.
25 serious adverse events (SAE) including 15 fatalities have been reported.
The results showed clearly that the new abridged 10-day melarsoprol treatment leads to comparable rates of cure and rates of serious adverse events.
The use of historic controls was problematic due to the observed underreporting of SAE. The average rate of encephalopathic syndromes was 11.6% which is higher than the 8% reported in the literature, but in the reported range of the historic controls of 11.4%.
The 10-day melarsoprol schedule against late stage *T.b. rhodesiense* sleeping sickness was shown to be non-inferior over the national treatment schedules and its introduction should be urgently considered as it improves the administration of medication and the treatment compliance.

1e

Three cases on Intestinal Capillariasis in Lao People's Democratic Republic

Phonepasong Soukhathammavong 1,2, Somphou Sayasone 1,2, Aphonethip Akkhavong 3, Aina Nirina Harimanana 3,Niranh Phommindh 4, Khamloun Choumalivong 5, Khamla Choumalyvong 5, Kongsap Akkhavong 2 , Michel Strobel 3, Christoph Hatz 1, Peter Odermatt 1

1 Swiss Tropical Institute (STI), Basel, Switzerland
2 National Institute of Public Health (NIOPH), Ministry of health, Vientiane, Lao PDR
3 Institut de la Francophonie pour la médicine tropicale, Vientiane, Lao PDR
4 Department of Internal Medicine, Setthathirath Hospital, Ministry of Health, Vientiane, Lao PDR
5 Department of Parasitology, University of Health Science, Vientiane, Lao PDR

Capillaria philippinensis is a rare zoonotic intestinal parasite that emerged in the 1960s. The outcome of intestinal capillariasis may be fatal if untreated in due time. We report the first cases from Lao People's Democratic Republic (Lao PDR). The three patients were unrelated previously healthy young men (24, 26, and 27 years) with no underlying disease or immune depression. Two of them acquired the infection in Thailand; the other patient had no travel history outside Lao PDR. All patients were seen several times in different hospitals before the diagnosis was made. All had concurrent parasites infection: *Giardia lamblia*, *Entamoeba histolytica*, *Strongyloides stercoralis*, *Opisthorchis viverrini*, and hookworm. The patients frequently consumed uncooked fish. After treatment with albendazole (400 mg/day for 21-30 days) all patients recovered. In Lao PDR, consumption of raw small freshwater fish is common. Therefore, the possibility for a capillariasis outbreak should be considered.

1f

Selection and application of synthetic peptides for serodiagnosis of helminth infections

LIST Claudia1, MÜLLER Norbert2, GOTTSTEIN Bruno2, FELGER Ingrid1

[1]Swiss Tropical Institute, Medical Parasitology and Infection Biology, Basel, Switzerland
[2]Institute of Parasitology, University of Bern, Bern, Switzerland

Diagnostic antigens currently used in helminth serology (enzyme-linked immunosorbent assay (ELISA) and Western blot) mostly originate from crude antigen extract of adult worms, larvae or eggs. Only few antigens are recombinantly produced. Moreover, established diagnostic tests are not satisfactory with respect to sensitivity and specificity and are dependent on availability and purity of antigen preparations. In order to resolve these limitations, we aim at the establishment of a bioinformatic selection procedure identifying antigenic peptide candidates by genome-wide analysis. Successful peptide candidates will be chemically synthesised and applied as standardized starting material for diagnostic assays.

Protein sequences of *Echinoccocus* spp*.* (Platyhelminthes, Cestoda) available in public databases were searched for unstructured and coiled-coil domains from secreted, transmembrane or membrane-anchored proteins. From the identified sequences, 30mer peptides were chosen according to stability and antigenicity predictions. These peptides were chemically synthesized, printed on micro-arrays and screened for reactivity with sera from helminth infected patients. Sero-reactive peptides were transferred to the ELISA platform and currently undergo testing for sensitivity and specificity.

The first round of serological screening on a micro-array platform provided positive results for *Echinococcus* spp.. In total, 16 out of 45 *Echinococcus* peptides showed reactivity with sera from echinococcosis patients. Only one of these 16 peptides was cross-reactive with negative control sera.

We have successfully obtained proof of principle for discovery of diagnostic peptides on a microarrray platform. The diagnostic analytes identified by our prototype microarray reacted with variable numbers of positive sera and should be used in combination to increase test sensitivity. Due to the restricted length of synthetic peptides on a microarray, they likely show reduced sensitivity compared to their full length recombinant counterparts. Therefore, the next step of development will assess the performance of combinations of several epitopes in a single assay.

Scientific session 2: Public Health

Chair: H.-P. Marti

2a

Swiss support to the national programme for insecticide-treated nets in Tanzania (NETCELL)

Christian Lengeler[1], Nick Brown[2]

[1]Swiss Tropical Institute, Basel, Switzerland; [2]National Malaria control Programme, Dar es Salaam, Tanzania.

Vector control is currently the most important preventive measure for malaria. Since 1998 (start of the Roll Back Malaria Partnership), there has been a rapid development of vector control on a large scale in many endemic countries, especially in sub-Saharan Africa. In most countries, insecticide-treated nets (ITNs) remain the method of choice for vector control, because ITNs are more cost-effective and feasible compared to other methods.

Since 2002 Switzerland is supporting the development of the national ITN programme in Tanzania. Major contributions were made to the design and the running of the programme, in collaboration with a large number of partners. With support from the Swiss Agency for Development and Cooperation (SDC), the Swiss Tropical Institute is running an ITN cell within the Tanzanian National Malaria Control Programme, with relevant professional staffing. During this period a national-scale public-private partnership was implemented, with a comprehensive distribution of nets and a voucher scheme to target pregnant women and small children.

The Swiss support has averaged CHF 600,000 per year for the period under consideration. However, this support has been instrumental in generating nearly USD 200 million in total funding for the ITN programme. This illustrates both the dramatic change in the fundability of malaria control programmes and the large leverage effect of the Swiss investment. During this period, household net ownership increased from below 30% to 56%, and insecticide-treated net ownership from 3% to 39%. Similarly, coverage in children under five years sleeping under a net increased from 20% to 36%. A large mass distribution of long-lasting insecticidal nets is planned for 2008-2009 and this should help to bring effective children coverage above 80%. Over this period child mortality has been reduced nationally by over 20% and at least half of this reduction should be due to malaria control.

The Swiss support to the national ITN programme in Tanzania has been crucial in funding and upscaling and it allowed it to grow to one of the most effective programmes in SSA. Over the two next years much higher coverage rates will be achieved and the current trend of reductions in mortality and morbidity due to malaria should continue.

2b

Innovations in improving access to malaria treatment in Tanzania: the ACCESS Programme

Christian Lengeler[1], Manuel Hetzel[1], Flora Kessy[2], Brigit Obrist[1], Angel Dillip[2], Sandra Alba[1], Ahmed Makemba[2], Christopher Mshana[2], Alexander Schulze[3], Hassan Mshinda[2]

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Access to appropriate and timely malaria treatment is hampered by inter-linked factors at household and health system levels. We assessed the relative importance of a range of potential obstacles to effective malaria treatment in rural Tanzania and developed multiple interventions to address them.

The ACCESS programme is based on a framework considering availability, accessibility, affordability, acceptability, accommodation and quality of care as central components of access. Our studies were conducted in the area of a Demographic Surveillance Site (DSS) with a total population of approximately 80,0000. In addition to this demographic monitoring, we used semi-quantitative cross-sectional community surveys to investigate disease perception and treatment seeking behaviour, complemented by quantitative and qualitative studies on drug availability, quality of care and private drug dispensing units.

Programme interventions included social marketing to increase appropriate malaria treatment and activities aimed at improving quality of case-management (including diagnostics) and the upgrading of rural drug shops.

Socio-cultural factors are not prominent obstacles to treatment-seeking any more, probably as the result of long-term health promotion. However, mobilizing adequate resources (in particular finances) to access care was consistently found to be a problem, especially since the exemption policy for children did not work. Health-facility attendance was very common for malaria in children. Children attended health facilities more frequently than adults (78 vs. 53%) but 30% of children and 41% of adults bought their antimalarials from shops as a result of frequent drug stockouts. Of 80 fever cases in children <5 years, 88% received a recommended antimalarial but a large proportion of treatments were wrongly dosed.

The ACCESS Programme is contributing steadily to the local, national and international research agenda on how best to use limited resources. While much can be done to improve the availability of malaria treatment in rural Africa settings, this is very much linked to the development of health services in general.

2c

Patient costs during the intensive and continuation phases of tuberculosis treatment in Central Asia

R Ayé,[1] K Wyss,[1] S Saidaliev,[2] H Abdualimova[3]

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[2]Republican Centre Against tuberculosis, Dushanbe, Tajikistan;
[3]Project Sino, funded by SDC, Dushanbe, Tajikistan

Tuberculosis puts a heavy economic burden on households. Patient costs of tuberculosis in the former Soviet Union, a setting with high informal payments, have hardly been studied. This study measured the costs of tuberculosis at the household level in Tajikistan.
Adult pulmonary tuberculosis patients who were registered in the 12 study districts over a four month period were enrolled. Each patient was interviewed twice, once during the intensive (IP) and once during the continuation phase (CP). Comprehensive information on direct costs (expenditure for drugs, medical services, transport, changes in expenditure for food, other non-medical items) and indirect costs (loss of income) was collected.
205 patients completed the first, and 143 patients both interviews. Mean total costs of tuberculosis at the household level were extrapolated to $1197. Direct costs amounted to $412 per patient, of which 34% ($140) were incurred before the tuberculosis diagnosis. Direct costs per month were $69 in IP and $27 in CP. Medical costs constituted 53% of total direct costs during treatment. Drug costs, mainly for vitamins and IV rehydration, made up 27% of total direct costs, while increased expenditure for food contributed 23% and transport 22%. Hospitalised patients' costs for drugs and transportation were more than double those of ambulatory patients.
The total costs of a tuberculosis episode at the household level amount to 350% of the per capita gross national disposable income (GNDI) of $342. The direct costs alone exceed per capita GNDI by 20%. The economic burden to households was most acute during IP, as monthly expenditure was more than twice as high during IP than during CP.
To enable tuberculosis patients to adhere to treatment, mitigation strategies are needed, especially during IP. Without such, tuberculosis will be hard to control.

2d

Water recontamination at point-of-consumption – improved supply sources and home-based water treatment will not guarantee safe drinking water

S. RUFENER[1,2], D. MÄUSEZAHL[2], H.-J. MOSLER[3], and R. WEINGARTNER[1]

[1] Group of Hydrology, Geographical Institute, University of Bern, Switzerland
[2] Swiss Tropical Institute, Basel, Switzerland
[3] Swiss Federal Institute of Aquatic Sciences and Technology, EAWAG, Dübendorf, Switzerland

Water-borne diseases are responsible for about 4 billion cases of diarrhoea every year, of which 2.2 million cases lead to death. The most important and immediate risks to human health are those from enteric microbes of faecal origin that are often transmitted by drinking water. Thereby, in-house contamination of drinking water plays an important role in developing countries.
The objective of our study was to identify critical points of water contamination along the potential transmission pathway from water source to drinking cup as well as to determine the extent of recontamination after water treatment. Totally, 81 households in rural and semi-urban Bolivia were visited and a total of 348 water samples of current water sources, transport vessel, treated water (45 households) and drinking vessels were analyzed. Water quality was assessed using *Escherichia coli* (*E. coli*) as indicator for faecal contamination. The concentration of *E. coli* increased significantly from water source (median = 0 CFU/100ml, interquartile range (IQR) = 0-13) to drinking cup (median = 8 CFU/100ml; IQR: 0-550). Boiling and solar water disinfection (SODIS) improved drinking water quality significantly (median = 0 CFU/100ml; IQR: 0-0.05); however, this quality improvement has often been reduced by recontamination at point-of-consumption, i.e. in drinking cups that are lifted to the mouth. Water recontamination occurred post-treatment in 16 out of 45 household that did apply a water treatment method. Even if the water quality at water sources is safe, it does not necessarily lead to good drinking water.
We conclude that interventions focusing exclusively on improvement of water sources and home-based water disinfection might not be effective without accompanying measures related to hygiene education and improved sanitation.

2e

Emergence of the eye worm *Thelazia callipaeda* in southern Switzerland and occurrence of its potential vector *Phortica* spp.

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Thelazia callipaeda is commonly known as the “oriental eye worm”. In Europe, *T. callipaeda* emerged after 1989 as parasite of dogs, cats, foxes and humans in Italy and in France. In southern Switzerland (Ticino), the first case of *T. callipaeda* in a dog was detected in 2000. Recent investigations identified *Phortica variegata* (Diptera, Drosophilidae) as a potential vector and intermediate host of *T. callipaeda*. Because of an increasing number of canine thelaziosis in Ticino, a retrospective survey in veterinary practices was carried out, and in 2005 all veterinarians in this canton were asked to report these cases using a questionnaire. Collected nematodes were morphologically and genetically identified as *T. callipaeda*. Since 2000, 129 *Thelazia*-cases were reported from the approximately 20’000 dogs living in Ticino, and 4 from cats. Seventy-two of these animals (59.5%) had never crossed the Swiss border. From July to September 2006, 529 randomly chosen dogs, to which an anaesthesia was administered, were additionally checked for the presence of adult specimens of *Thelazia*, and 28 of them (5.3%) were positive. *Thelazia*-infections were furthermore diagnosed in 7 of 126 foxes (5.6%) shot in Ticino between December 2005 to February 2006. Affected foxes, dogs and cats originated from the same regions. In addition, four sites in Ticino and one in Zürich were investigated in 2007 for the presence of *Phortica* spp. A total of 1695 flies were captured, mainly in the lowlands of Southern Ticino, but none was positive for *Thelazia* by PCR. The results indicate that thelaziosis is by now endemic in Southern Ticino. Since the insect vector is present also north of the alps, local outbreaks of the disease originating from parasites imported with returning travelling dogs cannot be excluded.

Scientific session 3: Parasitology

Chair: Hanspeter Beck

3a

Echinococcus multilocularis phosphoglucose isomerase (EmPGI): a moonlighting enzyme?

Britta Stadelmann, Urban Deutsch§, Bruno Gottstein*, Andrew Hemphill**

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For a better understanding of the host-parasite interaction in alveolar echinococcosis (AE), special attention has been given to the laminated layer (LL). This acellular and heavily glycosylated LL surrounds the entire metacestode, builds up the host-parasite interface, and it contributes critically in modulation of the physiological and immunological reactions on part of the host.

The laminated layer was purified from *in vitro* generated *E. multilocularis* metacestodes, and antisera were produced in rabbits. One of the respective reactive proteins associated with the purified LL and reactive with the antiserum is the enzyme phosphoglucose isomerase (PGI). Besides the LL, EmPGI is present in the germinal layer and in vesicle fluid. In general, PGIs are essential enzymes in glycolysis that are present in almost every cell. However, more recent findings have shown that other extracellular functional activities are carried out by PGIs: PGIs can act as a cytokines and growth factors and are known to enhance angiogenesis, tumor invasion and metastasis formation, all features that are also compatible with the growth characteristics of *E. multilocularis* metacestodes. *Echinococcus*-PGI (EmPGI) was cloned and functionally expressed in *E. coli*, and recombinant recEmPGI was characterized in terms of its glycolytic functional activity. Additionally, a putative extracellular role of recEmPGI as a cytokine was shown by stimulation of endothelial cell proliferation *in vitro*. A further study is now directed towards elucidating a putative autocrine role of EmPGI within the cyst, by stimulation of germinal layer cells.

3b
How *Giardia lamblia* deals with Stress

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Encystation comprises substantial modification of *Giardia lamblia* trophozoites. Cyst wall formation is the major phenotypic hallmark and includes synthesis, folding and secretion of cyst wall proteins (CWPs) which form a resistant matrix around the cell. *Giardia* lacks a classical Golgi and can only produce rudimentary core glycans for N-liked glycosylation. This is consistent with the absence of all factors involved in N-glycan-dependent quality control of glycoprotein folding and degradation in the ER. So far, only few homologs of the ~ 300 factors upregulated during the unfolded protein response (UPR) could be identified in the Giardia Genome Database (GGD). Specifically, key regulating factors which trigger UPR such as Ire1 and HAC1 are absent. The UPR is tightly regulated on the level of transcription and universally conserved among eukaryotes. This raised the question how *Giardia* deals with acute ER stress, as for example during the synthesis of large amounts of cyst wall proteins in the ER of early encysting cells.
Here, we investigated the giardial response to ER stress. Specifically, we asked which genes are upregulated on the transcriptional level in response to an unspecific stress (40^{o}C) or to redox stress affecting the secretory pathway. For the latter we mimicked overload of the ER with misfolded protein by treating trophozoites with the reducing agent dithiothreitol (DTT). The response was analyzed by transcriptional profiling using whole genome microarrays .Initial experiments showed a limited set of ~30 mRNAs that were significantly upregulated after 30 minutes. This set was moderately dose dependent and overlapped only in one case with mRNAs upregulated in response to heat stress. To further dissect the dynamics of the giardial redox stress response we performed time course experiments over 2 hours. We show that 1) redox stress indeed elicits a defined transcriptional response; however, 2) the number of upregulated genes is only one fifth of the yeast set of UPR genes, and 3) unlike the yeast UPR, which is constant over time, the giardial stress response is organized as two distinct “waves” of upregulated transcripts. Taken together the data indicate a fundamentally different organization of the giardial stress response when compared with the classical UPR which is not easily explained by reductive processes alone.

3c
Transcriptional profiling of differentiation in *Giardia lamblia*

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The protozoan *Giardia lamblia* is an intestinal parasite with a worldwide distribution and a major cause of diarrhoea in both humans and animals. *Giardia* has a simple two-stage life cycle consisting of the asexually dividing, flagellated trophozoite and the infectious cyst. *C*ysts can survive outside the host for months and uptake of less than 10 cysts is sufficient for a new infection.
Stage-conversion of trophozoites into cysts (encystation) can be induced *in vitro* and is completed within 20-24 hours. Encystation entails synthesis, maturation and regulated secretion of an extracellular matrix which polymerizes to a cyst wall conferring environmentally resistance to the parasite. This biopolymer appears to have a very simple composition. So far only three paralogous cyst wall proteins (CWP1-3) and a β-1-3 GalNac homopolymer have been identified as structural components. In this work, we aim to identify other hallmark factors of encystation with emphasis on the cyst wall components.
The expression of CWPs is transcriptionally regulated and upregulated shortly after induction of encystation. Therefore we hypothesize that expression of putative unknown components of the cyst wall are regulated in a similar fashion. To address this question, we performed transcriptional profiling of *Giardia* trophozoites post induction of encystation *in vitro*. Whole genome transcriptome analyses using TIGR microarrays and two-dye labelling were carried out to identify upregulated genes.
With this approach, we identified a two-phase induction of a limited set of genes with the known CWP genes being upregulated at 3h and 7h post induction of encystation. Altogether, the data confirm that this developmental step is defined by a limited set of transcriptionally regulated factors and that the extracellular matrix has a low complexity. Currently, we are investigating selected candidate structural proteins which may be involved in building or anchoring the cyst wall material.

3d

Neogenesis and maturation of a naturally pulsed Golgi-like organelle system during stage-differentiation in *Giardia lamblia*

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The protozoan parasite *Giardia lamblia* has a basic but effective trafficking system and lacks a classical Golgi apparatus with stacked cisternae. During evolution Giardia has undergone dramatic reduction of all organelle systems investigated to date, but still possesses sophisticated secretory and endocytic transport pathways. For transmission to new hosts, trophozoite stages differentiate to cysts by producing a massive extracellular matrix (cyst wall) composed of only three cyst wall proteins (CWPs) and a homopolymer glycan. CWPs are exported from the ER and sorted rapidly into encystation-specific vesicles (ESVs). Although ESVs have no similarity to the classical Golgi of higher eukaryotes, all available data suggest that they are developmentally regulated Golgi-like organelles. Because they contain a limited set of pre-sorted cargo proteins, ESVs may be unique post ER organelles arising de novo and undergoing synchronous cisternal maturation.

To test this hypothesis we probe the regulated secretory system using conditional expression of dominant-negative variants of small GTPases. The combined data shows that ESV neogenesis follows universally conserved principles and is remarkably similar to ER to Golgi transport despite a completely different morphofunctional organization. Time-lapse microscopy demonstrates that ESVs mature into an organelle network reminiscent of the Golgi ribbon. Quantitative analysis reveals rapid exchange of cargo via dynamic tubular connections. We also show processing dependent dense core formation in ESVs. This results in cargo sorting prior to regulated secretion of the cyst wall material in two stages, which is key to achieving environmental resistance necessary for transmission to a new host. The naturally pulsed nature of this Golgi-like export system in Giardia provides a unique model to study universally conserved processes in secretory granule formation in a highly simplified context.

3e
Essential role of P-gp in *Toxoplasma gondii* replication

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P-glycoprotein (P-gp) is a member of ABC transporters and its overexpression, following drug treatment, is responsible for multidrug resistance in tumor cells and in pathogenic organisms by ATP-driven efflux of drugs from the cell. Recently, sequences of a P-gp homologue have been identified also in *T. gondii*. However, the physiological functions of this protein in absence of drug pressure are still not completely understood.
We are investigating the role of P-gp in host-parasite interaction using the model parasite *T. gondii.* By using pharmacological inhibitors of P-gp and P-gp KO host cells, we showed that host cell P-gp is essential for optimal *T. gondii* replication and plays a role in the parasite cholesterol metabolism. The absence of the host P-gp compromised cholesterol uptake by intracellular *T. gondii.* We also showed that P-gp plays a role in cholesterol redistribution within the plasma membrane of host cells. In addition, absence of host cell P-gp affected replication of *Neospora caninum*.
The characterization of P-gp functions in infected host cells will further our understanding of host-parasite interaction and likely lead to the identification of novel essential processes for parasite survival and potential new drug targets.

3f
Toxoplasma gondii P-glycoprotein: insights into essential cellular functions

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P-glycoprotein (P-gp, ABCB1), a member of the ubiquitous superfamily of ATP-binding cassette (ABC) transporters, mediates the trans-membrane transport of structurally diverse agents ranging from ions to peptides. Importantly, P-gp actively exports xenobiotics from the cell and its elevated expression is associated with multidrug resistance in cancer cells and infectious diseases.
Recently, 24 ORFs containing motifs typical of ABC transporters were identified in the genome of *T. gondii* and these genes were shown to be transcriptionally active in both the tachyzoite and bradyzoite stages of the parasites. However, the molecular properties of *T. gondii* ABC transporters and the intracellular biochemical processes mediated by these proteins remain elusive. We started to analyze the *T. gondii* homologue of P-gp in terms of protein expression, localization and function. By using antibodies specific for *T. gondii* P-gp and pharmacological inhibitors we show that the protein re-localizes in the parasite during its intracellular lytic cycle and that P-gp activity modulates parasite replication and lipid metabolic processes.

3g

Neospora caninum and the interaction with murine bone marrow-derived dendritic cells

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Dendritic cells (DCs) are the first defence of the innate immune system after infection with pathogens. So far, nothing is known about the invasion and survival ability of *Neospora caninum* in mouse bone marrow DCs (mBMDCs), as well as cytokine expression pattern after DCs had contact with tachyzoites.
In the present study, we stimulated mBMDCs with live and different kinds of inactivated parasites (liquid nitrogen, 56°C for 50 min, or 1.5% PFA). Invasion and survival ability was determined by NcGRA2-RT-PCR and electron microscopy (SEM, TEM). Further, cytokine expression was evaluated by RT-PCR and cytokine-ELISA.
SEM and TEM of DCs stimulated with live parasites revealed that *N. caninum* was able to invade cells. Further on, the parasites could survive and proliferate in DCs, as proven by NcGRA2-RT-PCR. Cytokine expression analysis (by RT-PCR) exhibited that live and inactivated parasites stimulate DCs in a way that they express IL-12p40, IL-10 and TNF-a, whereas IL-4 levels were below the detection limit.
The work present here was a first approach to determine survival and proliferation ability of *N. caninum* in mBMDCs and cytokine expression pattern of DCs after stimulation with parasites. The fact that parasites survive inside DCs is important as DCs are competent immune cells that disseminate throughout the host body. Thereby, migrating DCs can transport tachyzoites from periphery to organs, as it was described for the related parasite *Toxoplasma gondii*.

Scientific session 5: Veterinary diseases

Chair: A. Mathis

5a

Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by in vitro cultivation and PCR

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Tritrichomonas foetus, a parasite well known for its significance as venerally transmitted pathogen in cattle, has recently been identified as a cause of chronic large-bowel diarrhea in domestic cats in the US, UK, and more recently also in Norway. Switzerland is currently considered as free from *T. foetus* in cattle, and the parasite has not been found in cats so far. The aim of this study was to establish diagnostic methods for this emerging pathogen and to screen cats for infection.

In a period of 3 months (October to December 2007), 45 cats of Switzerland suffering from chronic diarrhea were investigated for intestinal infections, including a search for trichomonads. A commercially available in vitro culture system was used to screen for infection, complemented with a PCR and subsequent amplicon sequencing to support speciation. The PCR is based upon amplification of a sequence derived from the internal transcribed spacer region 1 (ITS1) on the ribosomal RNA gene (rRNA) using primers designed to detect a broad range of genera and species belonging to the family of Trichomonadidae. The method was furthermore adapted to the uracil DNA glycosylase (UDG)-system in order to prevent carry-over contamination and it included a recombinant internal control to track for inhibitory reactions.

Eleven out of the 45 cats were culture-positive, as revealed by microscopic identification of trichomonadid organisms. One of the isolates was subjected to scanning electron microscopy and findings revealed the presence of three flagella, thus placing the isolate into the gender *Tritrichomonas* sp. PCR and subsequent amplicon sequencing were carried out with 10 of the 11 isolates. A total homology with published *Tritrichomonas foetus* sequences was confirmed in all of the cases.

T. foetus therefore appears to range among those organisms that can cause chronic diarrhea in cats in Switzerland.

5b

Validation of a Western Blot for the detection of anti-*Trichinella* spp. antibodies in domestic pigs

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Trichinellosis is a zoonotic disease in humans caused by *Trichinella* spp.. Measures to protect consumer health include testing pigs at slaughter for the presence of muscle stage larvae of *Trichinella* spp.. Also, the EU regulation as well as guidelines of the World Organisation for Animal Health (OIE) foresee the possibility of serological surveillance to demonstrate the absence of *Trichinella* spp. in a defined domestic pig population. Most ELISA tests presently available do not yield 100% specificity, and therefore there is a need for a complementary test to confirm the specificity of any initial ELISA-seropositivity. The goal of the present study was to evaluate the sensitivity and specificity of a Western Blot assay.

The Western Blot, using somatic *Trichinella spiralis* muscle stage (L1) antigen, was tested as a confirmatory method to validate seropositive ELISA findings in the framework of serological surveillance for *Trichinella* infections in domestic pigs. Bayesian modeling techniques were used to account for the absence of a true gold standard test, as well as to correct for conditional dependence between serological tests. A total of 295 *Trichinella*-larvae negative samples and 93 *Trichinella*-larvae positive samples were included in the study. The *Trichinella*-larvae negative samples included 74 potentially cross-reacting samples from pigs with known other nematode infections.

The diagnostic sensitivity and specificity of the Western Blot were 95.8-96.0% and 99.5-99.6%, respectively. It was also demonstrated that the diagnostic sensitivity of the routine artificial digestion test was below 100%, even when the larval density of the samples exceeded the limit of detection. A sensitivity analysis showed that the model outcomes were hardly influenced by changes in the prior distributions, providing a high confidence in the outcomes of the models.

This validation study demonstrated that the Western Blot is a suitable method to confirm samples that reacted positively in an initial ELISA.

5c

Distribution and abundance of biting midges, the potential vectors of bluetongue disease in Switzerland

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Biting midges (*Culicoides* spp.) are tiny flies which, in Northern and Central Europe, used to be perceived as nuisance pests and as causative agents of allergic dermatitis particularly of horses. However, indigenous species proved to be highly efficient vectors for the recently introduced bluetongue virus (BTV). As there is only scant knowledge on the biology of biting midges, no control strategies are feasible as yet. The aims of this project are to determine the distribution, abundance, and activity patterns of biting midges occurring in Switzerland, to investigate host preferences among the midges, to define their breeding habitats on the basis of soil characteristics and to determine vector competence and capability that will eventually lead to vector suitability maps on a national scale.
Midges are being collected with Onderstepoort blacklight traps on farms located in the 12 climate regions of Switzerland as defined by the Federal MeteoSwiss. The traps are operated all year round, overnight, once a week. Identification of *Culicoides* to species complex level is achieved by microscopy. Multiplex real-time PCR assays are being developed for rapid and unequivocal species identification.
First results show that *Culicoides* spp. are present in all climate regions. The highest abundance was observed near Basel (Dittingen, BL, 364 metre asl) with overnight catches yielding up to 19'000 *Culicoides* spp. (mostly Obsoletus complex). Surprisingly, midges of the Obsoletus and Pulicaris complex, both potential vectors of BTV, were also found at the highest trapping site (Juf, GR, 2126 metre asl). Preliminary results suggest that breeding habitats are situated near the farmsteads.
Whereas an epidemic outbreak of bluetongue disease in Switzerland could be prevented by the large-scale bluetongue vaccination program of 2008, the few recent cases of the disease mainly in non-vaccinated ruminate livestock indicate a further spread of BTV towards Central Switzerland. The great need to further study the biology of these vectors is also indicated by the potential incursion of other *Culicoides*-borne diseases (e.g. African horse sickness).

5d

Flight performance of the Asian tiger mosquito *Aedes albopictus* (Diptera: Culicidae)

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Approximately 30 years ago, *Aedes albopictus* was introduced to the USA from Asia, most likely by ships carrying tyres, and later also to Europe. In the Southeastern USA, *Ae. albopictus* has largely displaced the Yellow Fever mosquito *Ae. aegypti*. The objective of this study was to analyze the flight performance of *Ae. albopictus* and to compare it to that of *Ae. aegypti*. Such comparisons may contribute to understand the reasons that led to the dispersal of *Ae. albopictus*.

A flight mill system was used to measure the flight performance of individual females up to four weeks of age. During 16 hours, flight distance, time and speed of flight activity was recorded for starved, sugar-fed, and blood-fed females.

Independent of their nutritive state and age, the majority of females flew continuously for intervals of 1–5 h before taking a rest, whereas the remaining mosquitoes had flight intervals of less than 1 h. On the first day post eclosion, starved and sugar-fed females showed a similar low flight performance of ~0.4 km/16 h. Starved females, 3 days post eclosion, flew a longer distance (X = 0.7 km/16 h) than their 1 day old siblings. Sugar-fed females reached their maximal flight distance of ~3 km/16 h at 3 days post eclosion, and this remained unchanged in older insects. The flight performance of sugar- and blood-fed females varied only slightly, averaging distances of 2.5 – 4.0 km with maximal flights of up to 8.6 km/16 h.

Briegel and co-worker (2001) showed that sugar-fed females of *Ae. aegypti* flew average distances of 3–5 km/16 h and had maximal flights of up to 18 km/16 h. We conclude that for these two potential vectors, flight performance does not differ and does not provide further insight into the displacement of mosquito populations.

Briegel, H., Knüsel, I., Timmermann, S.E., 2001, *Aedes aegypti*: size, reserves, survival, and flight potential. Journal of Vector Ecology 26, 21-31.

5e

Ticks and tick-borne pathogens from wildlife in the Free State province, South Africa

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Currently, in Africa, the role of many wildlife species in the circulation of tick-borne pathogens is still fairly unknown. Objectives of this study were to obtain more information on the host and vector ranges, as well as on the geographic distribution of tick species and their associated pathogens of the genera *Babesia, Theileria, Anaplasma* and *Ehrlichia* in the Free State province in South Africa.
Fieldwork was performed from March to June 2006 in four nature reserves and one private farm. Ticks and tissue biopsies were collected from ten wildlife species. Animals were either anaesthetized, caught alive, or hunted. Ticks and tissue biopsies were analyzed for tick-borne pathogens by PCR, followed by Reverse Line Blotting (RLB) and sequencing.
Globally, 37/569 ticks belonging to 3/8 species as well as 11/114 tissue biopsies belonging to 3/10 host species were found infected by at least one pathogen. Gene sequencing showed high degree of homology with *Anaplasma bovis*, *A. marginale*, *Theileria separata* and *Theileria* sp. "Malelane sable antelope". New pathogen-tick or pathogen-host associations were observed for *A. marginale, A. bovis, T. separata* and *Theileria* sp. "Malelane sable antelope".
Rhipicephalus (*Boophilus) microplus*, the known vector of *B. bovis*, was found for the first time in the Free State Province and *R. appendiculatus* was collected in areas where it was not described before.
In this study, we obtained some evidence that pathogens known to infect domestic animals (*T. separata*, *A. marginale, A. bovis*) also infect wild ruminants and/or their associated ticks. This suggests that wild ruminants may act as reservoirs for these pathogens, which enhances the complexity of their transmission cycles in nature and increases the difficulties to control tick-borne diseases of livestock. This is also relevant for wildlife health and conservation.

Posters

P1

Echinococcus multilocularis vesicles impair co-stimulatory molecule expression on human myeloid dentritic cells and their T cell stimulatory effect

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As dentdritic cells (DCs) are powerful antigen presenting cells (APCs), we were interested if the parasite in the context of its survival strategies is metabolically altering DC functions and so consequently the following T cell response. In a first step we investigated potential differences between healthy blood donors and alveolar echinococcosis (AE) patients. For that we stimulated monocyte-derived DCs with LPS and tested them for their maturation and function status. The results show an impaired maturation and a hampered T cell stimulatory effect of AE-DCs. Another aspect was the reaction of DCs to the stimulation with living parasite vesicles: DCs were cultured with or without vesicles to induce differentiation of immature DCs. The experiment presents that AE-DCs showed a less maturated grade (looking at CD80 and CD83 expression) as DCs from control blood donors. Lymphocytes showed a reduced proliferation after contact with vesicle-treated DC. We conclude that parasite vesicles can metabolically impair cell-mediated immune response by suppressing or modulating the DCs maturation.

P2

Monkey malaria in a European traveller returning from Malaysia

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More than 26 *Plasmodium* species are known to infect primates, but only few reports of naturally acquired monkey malaria in humans have been published. This may be due to the fact that these species are easily confounded with the better known human species.
A 53-year Finnish male was traveling for four weeks in Peninsular Malaysia, including a visit in the jungle for five days. He did not notice any mosquito bites.
Three days after his return the patient experienced high fever (38.8°C) that resolved quickly. The next day he had fever again and sought medical care. After transferral to the Helsinki University Hospital a coinfection of *P. falciparum* and *P. malariae* was diagnosed. Oral quinine hydrochloride and doxycycline were given for 10 days.
A nested PCR yielded an amplicon with a sequence identical to two *P. knowlesi* sequences. Six other published sequences differed only by one nucleotide, while more than ten differences were seen in comparison to human *Plasmodium* species.
P. knowlesi was first described in 1931 in a long-tailed macaque and in 1932 experimentally shown to be infectious to humans. The first natural infection in humans was reported in 1965. No other reports were published until 2004, when a study on PCR-negative *P. malariae* cases in Sarawak showed that *P. knowlesi* caused 58% of the 208 malaria cases studied. Cases reported from China, Thailand, Philippines and Singapore show that *P. knowlesi* infections in humans are not restricted to Malaysia.
P. knowlesi infection should be considered in malaria patients who have a history of a travel to forested areas in SE Asia, especially if *P. malariae* is diagnosed.

P3

Arbovirus transmitted by mosquitoes in southern Switzerland: a potential risk?

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Commercial trade, travellers and climatic global changes are facilitating the introduction and establishment of exotic vectors of pathogens in the temperate zones. According to the European Network on Imported Infectious Disease Surveillance (TropNetEurop), some cases of Arbovirus diseases (West Nile fever, Chikungunya) were reported in European countries during the last years. Several mosquito species detected in Ticino are potential vectors of pathogenic Arboviruses.
We assessed the presence of Arbovirus in different species of mosquitoes collected in Ticino, with special emphasis on Phlebovirus (Rift Valley Fever virus, Sandfly fever virus, Toscana virus), Alphaviruses (Sindbis virus and Chikungunya virus), and Flavivirus (Yellow fever virus, Dengue viruses and West Nile virus). Viral RNA was extracted from mosquitoes (adults and eggs) collected between 2007 and 2008, transcribed in cDNA and detected by a multiplex PCR followed by specific nested-PCR.
A positive amplification was obtained only for Flavivirus in female adults of *Aedes vexans* and *Aedes cinereus/geminus* or in *Aedes albopictus* eggs. Sequences were related to those of Kamiti River and cell fusing agent viruses that have been described as “invertebrate host only” Flaviviruses. Among the species collected in Ticino, the Asian tiger mosquito, *Aedes albopictus*, is particularly interesting because it is the competent vector for the transmission of the Chikungunya virus. The real-time RT-PCR based on the gene E1 established to detect CHIKV in mosquitoes (adults and eggs) and in human blood samples could not show the presence of CHIKV in the mosquito samples collected.
The Arboviruses we could detect in some of the mosquito samples investigated belong to a group of Flavivirus not known to be pathogenic for humans.
Given the significance of some of the disease agents mosquitoes may carry, close monitoring in Ticino must be continued.