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Swiss Society of Tropical Medicine and Parasitology

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Vector-Borne Diseases in Switzerland
–
A Changing Picture?

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

Spiez, 28-29 October 2010

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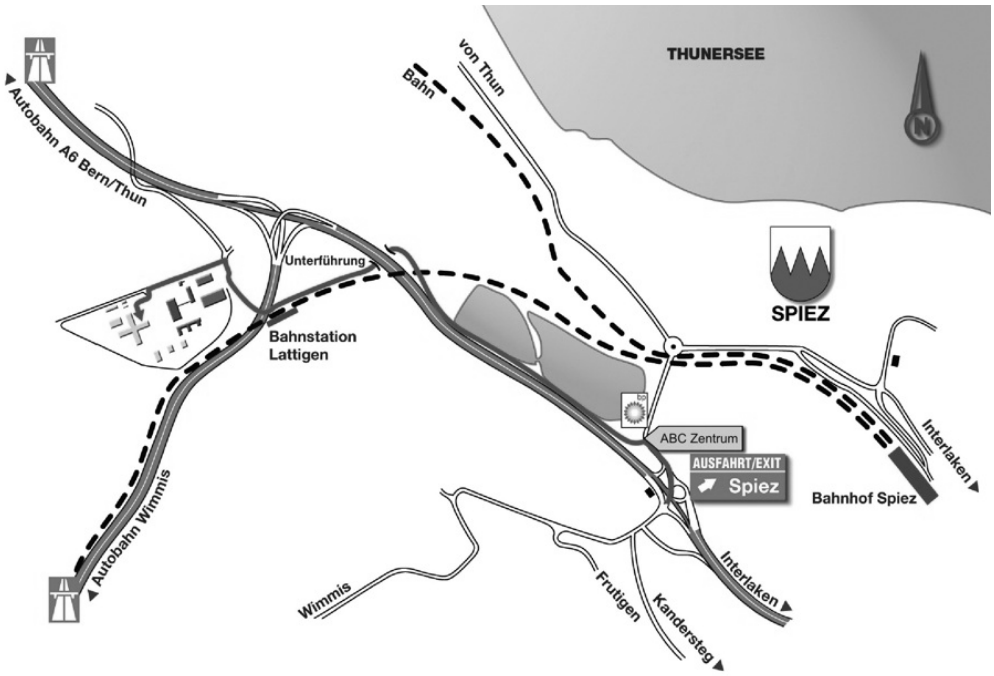

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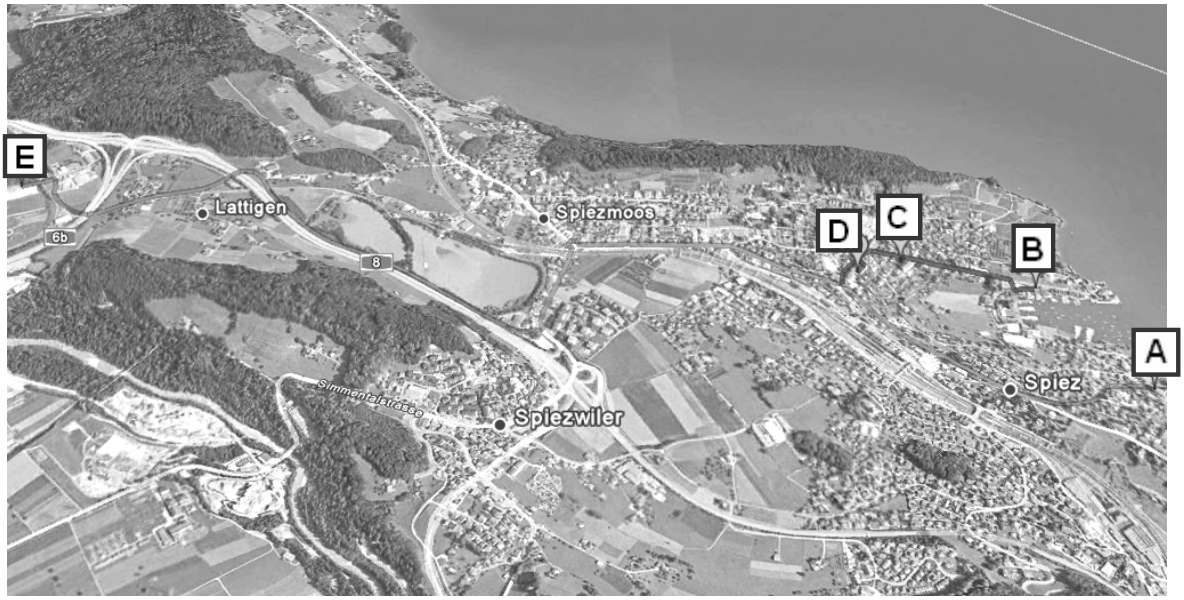

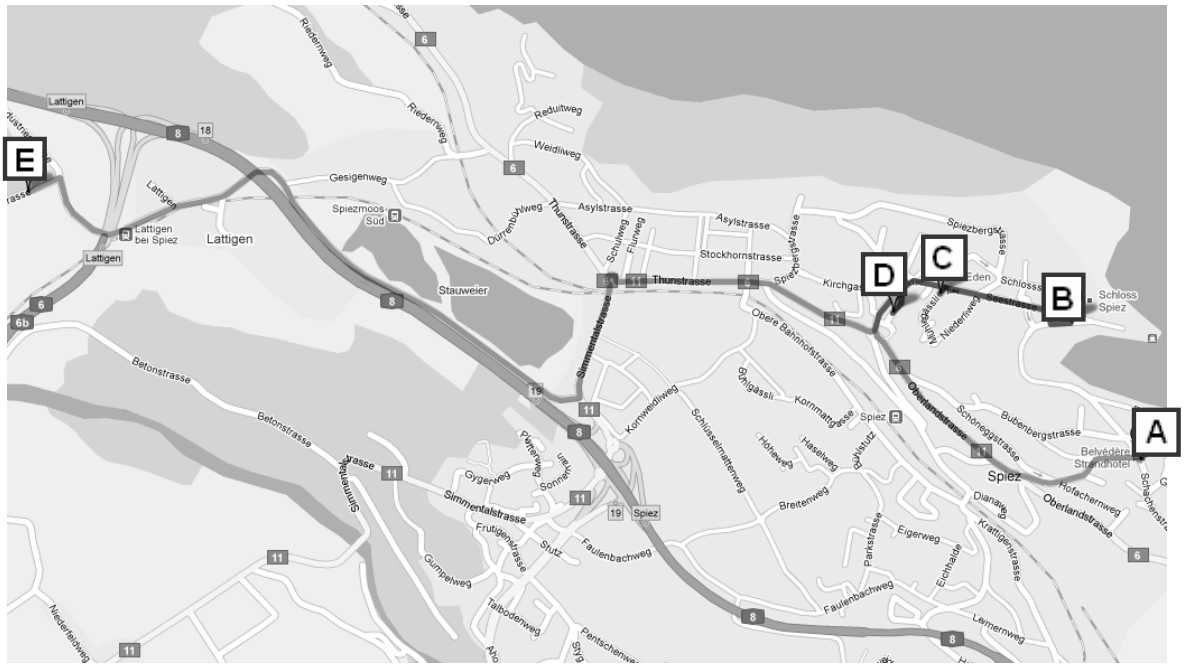

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Vector-Borne Diseases in Switzerland – A Changing Picture?

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

Spiez, 28-29 October 2010

Thursday 28th October 2010

Time	Title of session/presentation	Speaker
10.00-10.15	**Welcome** **C. Lengeler, President SSTMP** **A. Bucher, Head Strategy & Communication, Spiez Laboratory**	
10.15-11.00	***Keynote 1*** **Emerging vector-borne diseases in western Europe - do we need to be worried?**	**W. Takken** Wageningen
11.00-11.45	***Keynote 2*** **Spiez Laboratory: options for bio-defense and scientific research**	**M. Schütz** Spiez Laboratory
11.45-12.15	**Visit Spiez Laboratory (P3/P4 facilities)**	Staff Spiez Laboratory
12.15-14.00	**Lunch (cafeteria of Spiez Laboratory)**	
Scientific session 1:	**Veterinary Parasitology** Chairs: N. Müller, F. Grimm	
14.15-14.30	**Cutaneous bovine and equine leishmaniasis due to a yet non-specified *Leishmania* species**	**B. Gottstein**
14.30-14.45	**Genotypic and proteomic characterisation of *Tritrichomonas foetus* isolates from cats**	**K. Reinmann**
14.45-15.00	**New techniques for *Echinococcus multilocularis*: subcutanous mouse model, high-throughput drug screening assay and RNA-interference**	**M. Spiliotis**
14.00-14.15	**Diagnostic and molecular epidemiology of bovine tuberculosis in Chad**	**R. Ngandolo**
15.00-15.15	**Tuberculosis at livestock-human interface in the Pastoralist communities of Southern Ethiopia**	**B. Gumi**
15.15-15.30	**nn**	**nn**

Scientific session 2:	**Public Health and Epidemiology** Chairs: D. Mäusezahl, P. Odermatt	
14.00-14.15	**The mortality impact of the ACCESS programme in Tanzania**	**S. Alba**
14.15-14.30	**Combining interventions: improved stoves, kitchen sinks and solar disinfection of drinking water and kitchen clothes to improve home hygiene in rural Peru**	**S. Hartinger**
14.30-14.45	**A prospective feasibility study on rapid diagnostic test & unified artemether/ lumefantrine-based treatment for malaria in Papua New Guinean infants exposed to *P. falciparum and P. vivax***	**N. Senn**
14.45-15.00	**Severe schistosomiasis mekongi in Southern Lao People's Democratic Republic**	**P. Soukhathammavong**
15.00-15.15	**Control of vector borne diseases in pets in Switzerland: an ESCCAP action**	**P. Deplazes**
15.15-15.30	**nn**	**nn**
15.30-16.00	**Tea break**	
16.00-16.45	***Keynote 3*** **The risk of Chikungunya outbreaks in Italy**	**G. Rezza / L. Busani** ISS Rome
16.45-18.00	**General Assembly of Swiss Society of Tropical Medicine and Parasitology (see separate agenda)**	
19.30	**Dinner at Hotel Eden** **(optional but booking required)**	

Friday 29th October

Time	Title of session/presentation	Speaker
08.30-09.15	***Keynote 4*** **Burden of Disease of zoonotic diseases**	**P. Torgerson** Zürich
09.15-10.00	***Keynote 5*** **Control of Aedes mosquitoes in Ticino**	**O. Petrini** Bellinzona
10.00-10.30	**Tea break**	
Scientific session 3:	**Human Parasitology** Chairs: HP. Beck, C. List	
10.30-10.45	**The survival of *Ixodes ricinus* (Acari: Ixodidae) under challenging conditions of temperature and humidity is influenced by *Borrelia burgdorferi* sensu lato infection**	**C. Herrmann**
10.45-11.00	**Synthetic peptides for the diagnosis of human echinococcosis**	**C. List**
11.00-11.15	**Molecular approaches and functional analysis of potential drug target proteins in *Giardia lamblia***	**D. Nillius**
11.15-11.30	**Arginine deiminase and ornithine carbamoyl transferase as pathogenicity factors of giardiasis**	**B. Stadelmann**
11.30-11.45	**First case of *Anaplasma phagocytophilum* seroconversion and seroepidemiology in Northern Switzerland**	**M.-L. Tritten**
11.45-12.30	**Two best presentations at the doctoral students meeting**	**nn**
Scientific session 4:	**Clinical Research & Public Health** Chairs: Ch. Lengeler, J. Lindgren	
10.30-11.00	**Etiology of fever in children from urban and rural Tanzania**	**V. D'Acremont (*SSTMP awardee)***
11.00-11.15	***Strongyloides stercoralis*: methods of detection and efficacy of treatment in school children in Cambodia**	**V. Khieu**
11.15-11.30	**Landscape Genetics Applied to Environmental Changes Impact on epidemiological systems in vector borne diseases: the Chagas Disease model in Brazilian Amazon**	**M. Quartier**
11.30-11.45	**Agreement among various NSD diagnoses**	**V. Paralikar**

11.45-12.00	**Tolerance and feasibility of Nifurtimox-Eflornithine Combination Therapy (NECT) for second stage *T. b. gambiense* HAT in Doruma, north-east DRC**	**D. Schrumpf**
12.00-12.15	**Sero-epidemiological survey of gnathostomiasis in Lao PDR**	**Y. Vonghachack**
12.15-12.30	**nn**	**nn**
12.30-14.00	**Lunch (cafeteria of Labor Spiez)**	
Scientific session 5:	**Entomology** Chairs: A. Mathis, P. Müller	
14.00-14.15	**Microclimate and zoonotic cycle of tick-borne encephalitis virus in a risk area in Switzerland**	**C. Burri**
14.15-14.30	**MALDI-TOF MS for characterization of *Culicoides* biting midges (Diptera: Ceratopogonidae)**	**C. Kaufmann**
14.30-14.45	**An *in vitro* assay for testing mosquito repellents that employs a warm body and carbon dioxide as a behavioural activator**	**Th. Kröber**
14.45-15.00	**Personal protection measure against ticks: *In vitro* and *in vivo* tests for the evaluation of active ingredients in tick repellent consumer products**	**Th. Kröber**
15.00-15.15	**Prevalence of zoonotic pathogens in questing *Ixodes ricinus* ticks in western Switzerland**	**E. Lommano**
15.15-15.30	**Identification of *Anopheles* mosquito species by molecular protein profiling**	**P. Müller**
Scientific session 6:	**Clinical case discussions** Chairs: F. Chappuis, F. Lüthi	
14.00-15.30	**See separate programme**	
15.30-16.15	**Keynote 6** **Tick borne diseases in Switzerland**	**O. Péter** **Sion & Neuchatel**
16.15	**Closure of meeting**	**C. Lengeler**

Scientific session 1:
Veterinary Parasitology
Chairs: N. Müller, F. Grimm

Cutaneous bovine and equine leishmaniasis due to a yet non-specified *Leishmania* species

Bruno Gottstein[1], Lisbeth Lobsiger[1], Caroline F. Frey[1], Wolf von Bomhard[2] Monika Welle[3], Norbert Müller[1]*

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Introduction
Recently, we identified a novel etiological agent of cutaneous leishmaniasis in a Swiss cow and in horses that, for most cases, sporadically appeared as autochthonous infections in geographically distant regions of Germany and Switzerland.

Methods
The infection was initially diagnosed upon clinical and immunohistological findings, complemented by electron microscopy. The histopathology of the lesions of the affected cow revealed that the epidermis of the animal was severely ulcerated and covered by a thick serous-fibrinous crust and, focally, few parakeratotic lamellar cell sheets. The dermis was diffusely infiltrated by numerous eosinophils and foamy macrophages. Few plasma cells, lymphocytes and a moderate fibroblast proliferation were visible. An inflammatory reaction extended deeply into the panniculus. The inflammation caused severe damage to local tissues, obliterating adnexal structures. Conventional in vitro cultivation of the *Leishmania* organisms failed so far. Subsequent comparative sequence analysis of *Leishmania*-PCR products (GeneBank™ accession numbers for the cow GQ281282, for the horses GQ281278, GQ281279, GQ281280, and GQ281281) from the internal transcribed spacer 1 (ITS1) of ssrRNA classified the respective *Leishmania*-isolates as neither Old World nor New World *Leishmania* species.

Key Results
The PCR-typed isolates exhibited a close phylogenetic relationship to *Leishmania* sp. *siamensis*, an organism recently identified in a visceral leishmaniasis patient from Thailand.

Discussion
The potential transmitting vectors for all these cases have not yet been identified. Future investigations will have to elucidate the veterinary-epidemiological relevance of this etiological agent, as well as biological parameters such as transmission mode and geographical origin and distribution.

References
1. Müller N, Welle M, Lobsiger L, Stoffel MH, Boghenbor KK, Hilbe M, Gottstein B, Frey CF, Geyer C, von Bomhard W: Occurrence of *Leishmania* sp. in cutaneous lesions of horses in Central Europe. Vet Parasitol. 166: 346-351 (2009).
2. Lobsiger L, Müller N, Schweizer T, Frey CF, Wiederkehr D, Zumkehr B, Gottstein B. An autochthonous case of cutaneous bovine leishmaniasis in Switzerland. Vet Parasitol. 169: 408-414 (2010).

Genotypic and proteomic characterisation of *Tritrichomonas foetus* isolates from cats

K. Reinmann[1], D. Leitsch[2], N. Müller[1], J. Müller[1], B. Gottstein[1], C. F. Frey[1]

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[2]Departement of specific Prophylaxis and Tropical Medicine, Medical University, Vienna, Austria

INTRODUCTION: *Tritrichomonas foetus*, the infectious agent causing veneral disease in cattle, was not detected in cattle in Switzerland for more than a decade. However, trichomonads that cannot be distinguished from bovine *T. foetus* by morphology or PCR and sequencing on the ITS-1 locus have been isolated from cats suffering from chronic diarrhea in the U.S., Europe and Australia. A recent study performed in Swiss cats suffering from diarrhea revealed a high prevalence of 24% of trichomonad-infections (Frey et al., 2009). *T. foetus* in cattle is a notifiable disease and therefore the question arises whether bovine and feline isolates belong to the same species.

METHODS: A new PCR targeting Elongation Factor 1 (EF1) of trichomonads was established. Trichomonad isolates from cat faeces were analysed on two distinct genome loci (ITS-1 and EF1) and sequences were compared with the bovine *T. foetus* reference isolate as well as with *Trichomonas gallinae, Tetratrichmonas gallinarum, Trichomonas vaginalis, Pentatrichomonas hominis, Tritrichomonas mobilensis* and *Tritrichomomnas suis*. Furthermore, the protein expression of a feline and the bovine isolate were compared using 2D-gels.

RESULTS: Molecular analysis of two independent gene-loci showed complete homology between the feline and the bovine isolates of *T. foetus*. *T. suis* and *T. mobilensis* differ only in single nucleotides from the *T. foetus* sequence. *P. hominis, T. vaginalis, T. gallinarum* and *T. gallinae* however are clearly distinguishable from the former isolates at both loci. 2D-gel analysis revealed a very similar pattern for both the feline and the bovine isolate, but some subtle differences could be observed and may be worth investigating in future.

DISCUSSION: Our results provide further evidence that bovine and feline *T. foetus* isolates do belong to the same species. However, we want to strengthen this data by including more field isolates from bovines in the near future. Furthermore, the cattle population of Switzerland, mainly the animals used for natural breeding, need a close monitoring to make sure that a re-emergence of *T. foetus* in cattle in Switzerland would not remain undetected.

Ref: Frey CF, Schild M, Hemphill A, Stünzi P, Müller N, Gottstein B, Burgener IA., 2009: Intestinal *Tritrichomonas foetus* infection in cats in Switzerland detected by in vitro cultivation and PCR. Parasitol. Res., Mar; 104(4):783-788

New techniques for *Echinococcus multilocularis*: subcutanous mouse model, high-throughput drug screening assay and RNA-interference

Markus Spiliotis[a], *Chiaki Mizukami*[b], *Tatiana Küster*[a], *Corina Herman*[a], *Andrew Hemphill*[a], *Bruno Gottstein*[a]

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Introduction: One of the best characterized model organism for cestodes with human relevance is *Echinococcus multilocularis*. The genome is fully sequenced and next to an intraperitonal mouse-infection model, methods for the *in vitro* cultivation of the infection-relevant metacestode larval stage or primary cells are established.

Methods/Results: Recent improvements in the methodology of isolating *Echinococcus* primary cells resulted in highly viable primary cell mini-aggregates which could be genetically modified by siRNAs showing a gene knock-down on the transcriptional and the translational level by RNA-interference. Additionally the cultivation of these mini-aggregates could be downscaled to the 96-well format with survival rates of more than ten days allowing now to perform drug screening assays on high-throughput levels. Since drug treatment studies on laboratory animals using the intraperitoneal infection model does not allow to monitor the parasite growth during the experiment, a subcutanous infection model was established. In this model, the size development of the growing parasite can be visually followed and drug-derived effects can thus be easily detected at an early stage.

Conclusion: Taken together, the described methods will represent helpful tools in studies concerning drug discovery or the biological development of *Echinococcus multilocularis*.

Diagnostic and molecular epidemiology of bovine tuberculosis in Chad

B.N. Ngandolo[1], C. Diguimbaye-Djaibé[1], B. Müller[2], L. Didi[1], M. Hilty[2], I. Schiller[3], E. Schelling[2], B. S. Toguebaye[4], A. J. Akakpo[5], J. Zinsstag[2]

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[2] Swiss Tropical and Public Health Institute, Basel, Switzerland
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Bovine tuberculosis (BTB) is a chronic infectious disease, transmissible to humans and many animal species. It is due to an intracellular infection of *Mycobacterium bovis* belonging to the *M. tuberculosis* Complex. It represents a major threat to cattle in developing countries, including Chad. In Chad, where the assessment of its impact on the national economy has not yet been done, we anticipate that the losses in tonnes of condemned meat are substantial.

This survey was done in the southern area of the country (Sarh and Moundou) and involved sampling of 954 cattle prior to slaughter. Cattle belonged to 3 local breeds (Arab, Mbororo or Fulani, Bogolodjé) and crossbreeds of these. Clinical examination of live animals was performed and blood samples were collected for the genetic characterization of breeds and detection of antibodies against *M. bovis* (Fluorescence Polarization Assay (FPA) and Single Intradermal Comparative Cervical Tuberculin (SICCT) test). The post mortem diagnoses consisted of slaughterhouse inspection as well as bacteriological and molecular diagnostic examinations.

Ninety-five of 929 Arab and Mbororo cattle were tuberculin reactors (10.3%). However, meat inspection of 919 carcasses at the slaughterhouse resulted in full or partial condemnation of 109 carcasses due to tuberculous lesions (11.8%). Amongst 108 carcasses, 120 samples were taken from organs with lesions. The smears from sampled tissues were stained with the Ziehl Neelsen stain for microscopic evaluation.

After combining results from the SICCT and smear microscopy, the prevalence of suspected BTB animals was estimated to be 14% (130 of 929 cattle). The cultures of 112 tissue samples revealed that 50 cattle were infected with acid-fast bacilli. The real-time PCR showed the presence of mycobacteria in 33 cattle, with 13 isolates being non-tuberculous mycobacteria and 20 isolates belonging to the *M. tuberculosis* complex. Spoligotyping further showed that 13 of 108 condemned carcasses (12%) were infected with *M. bovis*. The spoligotype patterns of all *M. bovis* isolates lacked spacers 3, 9, 16 and 30. The lack of spacer 30 was previously reported as typical for *M. bovis* strains in Central Africa, which is also characteristic of *M. bovis* BCG. This suggests a European origin. This is the first description of *M. bovis* in Southern Chad.

The assessment of the techniques used for the detection of BTB in the population showed that the threshold used for the SICCT test should be decreased from > 4mm (current OIE recommendation) to ≥ 2 mm to increase its sensitivity. We found no evidence that lesion positive animals not reacting to the SICCT (likely due to anergy) would be detected with the ante-mortem FPA test. The more expensive FPA test did not perform better than the SICCT. Therefore, the latter remains the standard test for detection of BTB in Chadian cattle.

Tuberculosis at livestock-human interface in the Pastoralist communities of Southern Ethiopia

Balako Gumi [a,c], *Esther Schelling* [b], *Abraham Aseffa* [c], *Douglas Young* [d], *and Jakob Zinsstag* [b]

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Introduction

Globally, tuberculosis (TB) causes 2 millions deaths per year and 8 millions people with disease. The proportion of cases due to *M. bovis* is unknown. Bovine tuberculosis (BTB) is endemic in cross-breed dairy farms and zebu cattle in the central highlands of Ethiopia. The information on human and animal BTB is scarce in pastoral settings. To determine prevalence of BTB in humans and livestock in the pastoralist communities of Oromia and Somali regions in the south Ethiopia, epidemiological study was carried out during 2008-2010.

Methods

The tuberculin skin testing was conducted on randomly selected 125 herds comprising 59 cattle, 32 camels and 34 goats. A total of 1891 animals with 894 cattle, 479 camels and 518 goats were tested. Abattoir specimens from livestock, sputum samples from humans with pulmonary TB and fine needle aspirates (FNA) from humans with TB lymphadenitis were collected and processed at Armauer Hansen Research Institute. Culture positive isolates were characterized by deletion typing.

Results

The prevalence of BTB reactors in tested herds were 4.7% in cattle, 0.4% in camels & 0.2% in goats. The molecular characterization of 29 mycobacterial isolates from livestock revealed that 23 isolates from cattle were all *M. bovis*, of 2 isolates from camels one was *M. tuberculosis* while the other one was non–MTC and all of 4 isolates from goats were non–MTC. Out of 120 isolates from sputum samples 2.5% was *M. bovis* while the remaining was *M. tuberculosis.* This is the most important finding that confirms human-animal transmission of *M. bovis* in this study area. All 6 isolates from FNA samples were *M. tuberculosis*.

Discussion

Results from our study showed that the prevalence of TB due to *M. bovis* was 2.5% in human pulmonary TB and *M. bovis* infection was more prevalent in cattle than in camels and goats. In the future, further detailed study is needed to understand determinants of *M. bovis* transmission between animals and humans.

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Scientific session 2:
Public Health and Epidemiology
Chairs: D. Mäusezahl, P. Odermatt

The mortality impact of the ACCESS programme in Tanzania

Sandra Alba[1,2], Rose Nathan[2], Mathew Alexander[2], Manuel W Hetzel[3], Angel Dillip[2], Alexander Schulze[4], Flora Kessy[2], Hassan Mshinda[5], Christian Lengeler[1]*

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2 Ifakara Health Institute, Ifakara, Tanzania
3 PNG Institute of Medical Research, Goroka, Papua New Guinea
4 Novartis Foundation for Sustainable Development, Basel, Switzerland
5Tanzanian Commission for Science and Technology, Dar es Salaam, Tanzania

Background
The ACCESS Programme was implemented between 2004 and 2008 in the Kilombero and Ulanga Districts of south-central Tanzania to improve access to malaria treatment with a set of interventions targeting users and providers. This paper aims at assessing the ACCESS programme's impact on mortality.

Methods
The local Demographic Surveillance System (DSS) provided monthly and yearly estimates of under-five, infant and child mortality rates expressed as cases per 1000 person years (c/1000py) between 1997 to 2008. We used Poisson regressions to assess the impact of the ACCESS interventions accounting for the effect of other malaria interventions and contextual factors. We also attempted to understand the relative contribution of malaria risk and food availability to monthly variations in mortality.

Results
Under five mortality decreased from an average of 28.4 c/1000py in the years before 2004 to 18.9 c/1000py in 2008 but this was part of a longer secular trend dating back to 1997. The ACCESS interventions and Insecticide Treated Nets (ITN) coverage were independently associated with decreases in mortality, accounting for the effect of other malaria interventions and contextual factors (ACCESS: IRR comparing before 2004 vs. 2008=0.83, 95%CI=0.68 to 0.99; ITNs: IRR for every 10% increase in net ownership=0.97, 95%CI=0.95 to 0.98). Decreases in under five mortality were largely driven by decreases in infant mortality while child mortality (ages 1-4 years) remained constant. Infant deaths were more likely to occur in months of high transmission whereas child deaths were more frequent in months of low household food availability.

Conclusions
Improving access to malaria treatment appears to be a successful strategy along with increasing ITN coverage. Most of the decrease in under-five mortality has been driven by decreases in infant mortality. Little gains have been made in child mortality, which appears to be mainly driven by household food insecurity. Child survival programmes should recognise the important contribution of malnutrition to morbidity and mortality.

Combining interventions: improved stoves, kitchen sinks and solar disinfection of drinking water and kitchen clothes to improve home hygiene in rural Peru

S.M. Hartinger [1,2,3], *C.F. Lanata* [1,4], *A.I. Gil*[1], *Theresa J Ochoa*[5], *J. Hattendorf*[2,3], *D. Mäusezahl*[2,3]

[1] Instituto de Investigación Nutricional, Lima Peru
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Introduction: Today, 8.8 million children die each year, 18% of these deaths attributed to pneumonia and 15% to diarrhoea, amounting to a total of 2.9 million deaths in 2008. Simple but effective interventions that are advocated in rural areas to reduce a variety of diseases could help decrease this burden. The combination of different interventions might have synergistic effects on health improvement and cost effectiveness. However, it is crucial to ensure cultural acceptance of these interventions. The aim of the study is to develop an effective and culturally accepted home-based intervention package to reduce diarrhoea and lower respiratory illnesses in children.

Methods: We evaluated the performance and acceptance of cooking devices, household water treatments (HWT) and home- hygiene interventions, with qualitative and quantitative methods. We established the sources of contamination of child's food and drinking water at household level by analyzing a total of 275 samples in 64 rural households. Additionally diarrhoeagenic *E. coli* was isolated from all samples with total coliforms using a multiplex PCR. Stoves performance was assessed by using two standardized protocols -control cooking test and the kitchen performance test. Additionally, we collected user satisfaction and perceptions of health and illness and conducted in-depth interviews one year after the interventions were installed.

Results: In-depth interviews on hygiene improvement revealed a high demand for kitchen sinks, which were also included as part of the intervention. After one year of installation the improved stoves and kitchen sinks were all use. The new ventilated OPTIMA stove designs reduces time spend in the kitchen by 25% and daily wood consumption by 16%. Solar water disinfection as HWT was selected by the majority of the participants in a blind tasting. *E. coli* was found in 48% of drinking water samples and in 42% of kitchen wipes and was more frequent in caregivers' hands (29%) than on children's' (18%). Diarrhoeagenic *E. coli* was found in 33% of drinking water samples and in 27% of child meals.

Conclusion: The intervention package was successfully adapted to local customs, kitchen-, home- and hygiene management. High user satisfaction was primarily driven by convenience gains due to the technical improvements and only secondarily by perceived health benefits.

A prospective feasibility study on rapid diagnostic test & unified artemether/ lumefantrine-based treatment for malaria in Papua New Guinean infants exposed to *P. falciparum and P. vivax*

Nicolas Senn[1, 2, 3], Patricia Rarau[2], Ivo Mueller[2], Doris Manong[2], Mary Salib[2], John Reeder[4], Stephen Rogerson[3], Blaise Genton[1]*

[1] Swiss Tropical and Public Health Institute (Swiss TPH), Switzerland
[2] Papua New Guinea Institute of Medical Research, Papua New Guinea
[3] University of Melbourne, Australia
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Introduction: In malaria endemic areas, the management of febrile children relies on a quick attendance to get tested for malaria [Rapid Diagnostic Test (RDTm) or blood slide (BS)] and receive efficient malaria treatment only if positive. There are safety concerns about withholding antimalarial drugs from children with negative tests as well as no evidence of ACT effectiveness in areas with mixed endemicity [*Plasmodium falciparum* (Pf) and *P. vivax* (Pf)]. This study explores the feasibility of this approach in Papua New Guinea (PNG).

Methods longitudinal prospective study performed in outpatient clinics (Madang and Sepik, Papua New Guinea) with1605 infants 3 months old enrolled and followed-up for 2 years

Results Among 7211 febrile episodes, 5664 fulfilled the inclusion criteria. The mean duration of symptoms was 2.7 days (95%CI 2.6-2.8). 3940 (70%) had a negative RDTm. Among them, 142 (3.7%) re-attended the clinic within 7 days for fever, 1 died of lower respiratory tract infection (LRTI) with negative RDT & BS. 25 (0.6%) infants re-presented with a serious adverse event: 1 severe *Pv* malaria, 2 *Pv* malaria & LRTI, 19 LRTI and 3 alternative diagnoses. Of these, 24 were cured and 1 died of secondary meningitis (RDT and BS negative). There were 1724 positive RDTs. All infants were treated with Coartem®. 35 (2%) re-attended within 7 days for fever, none died and 6 (0.3%) developed a serious adverse event, ascribed to malaria in 5 children, and 1 infant with an alternative diagnosis.

Conclusion This study provides solid evidence that an effective health care access associated with malaria rapid diagnostic testing and efficient unified antimalarial treatment with ACT for positive cases is safe and feasible in infants in countries with limited resources and a high endemicity for both *Pf* and *Pv* infections.

Severe schistosomiasis mekongi in Southern Lao People's Democratic Republic

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Background: In 2007, within the context of a community-based survey on helminth infections in three villages we identified severe cases of schistosomiasis in Southern Lao People's Democratic Republic (Lao PDR).

Methodology/Principal Findings: We followed the patients for three years. Mean age of the nine patients was 36 years (range: 5 - 66 years); the sex ratio was 7:2; 7 patients were male. The leading clinical features were cachexia, hepatosplenomegaly, ascites, splenic varices and rupture of oesophageal varices. Patients were co-infected with *Opisthorchis viverrini* (n=6), Strongyloides stercoralis (n=1) and hookworm (n=7). Three years follow-up after praziquantel showed improvements in three of them (case 5, 6, 9), two adult patients (case 2, 3) remained unchanged or the status worsened. Two patients (case 4, 7) died due to oesophageal bleeding. Two new patients were diagnosed in 2009 (case 7, 8). Ultrasonography examination showed the formation of meandering splenic varices; splenomegaly remained unchanged over time; subclinical liver pathology and portal vein thickening were only slightly changed in adult patients; improvement after treatment was seen particular in young patients.

Conclusions: The presence of young patients documented that schistosomiasis transmission is currently still ongoing in Southern Lao PDR. A long-term control intervention including access to treatment, health education, sanitation and infrastructure is urgently required.

Control of vector borne diseases in pets in Switzerland: an ESCCAP action

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The European Scientific Counsel Companion Animal Parasites (ESCCAP) was formed in 2005 with the objective of developing guidelines for the control of major parasitic infections in companion animals. This will contribute to protect the health of pets, to reduce the risk of the public for contracting zoonotic diseases and to preserve the bond between pets and people. The guidelines are continuously subjected to regular revision in dialogue with practitioners and researchers across Europe, in respect of the great diversity of parasites affecting animal health and their frequent zoonotic impact. Guidelines and other information for vets and pet owners are presented on the website (www.esccap.org, for Switzerland www.esccap.ch). The guideline No. 5 'Control of Vector-Borne Diseases in Dogs and Cats' was thus launched on the website in 2009. This guideline identifies the "key" VBD groups that cause severe illness and/or pose a zoonotic risk, and which exhibit a high prevalence in some or all areas of Europe including Switzerland: Leishmaniosis mainly imported from Southern Europe, Babesiosis spreading eastwards from Western-Switzerland, Ehrlichiosis/Anaplasmosis and Dirofilariosis as well as Thelaziosis (caused by the eye worm *Thelazia callipaeda*) emerging predominantly in southern Switzerland. The guideline deals with the geographical distribution of the parasites, prevention by animal management and treatment. ESCCAP Europe is an independent, non-profit organisation registered in the UK. ESCCAP-CH is a working group of the Swiss Association for Small Animal Medicine and the Swiss Society of Tropical Medicine and Parasitology and several other national associates and delegates have agreed a partnership.

Scientific session 3:
Human Parasitology
Chairs: HP. Beck, C. List

The survival of *Ixodes ricinus* (Acari: Ixodidae) under challenging conditions of temperature and humidity is influenced by *Borrelia burgdorferi* sensu lato infection

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Introduction The hard-bodied tick *Ixodes ricinus* and *Borrelia burgdorferi* sensu lato (s.l.), the etiological agent Lyme borreliosis, have been thoroughly studied on their own but knowledge on the influence of infection by *Borrelia* on ticks remains scarce. The current study aimed at determining whether *Borrelia burgdorferi* sensu lato (s.l.) influence *I. ricinus* survival under thermo-hygrometric stress.

Methods *I. ricinus* questing ticks were tested under various values of relative humidity (13%, 32%, 51.5%, 61%, and 89% RH) at two different temperatures (12.5°C and 25°C) and investigated for *Borrelia* infection by real-time polymerase chain reaction and reverse line blotting.

Results The thermo-hygrometric factor that most importantly determined survival was saturation deficit (SD). As SD increased, tick survival rate decreased in all stages. Survival rate of females was highest (77.6%), followed by males (51.6%), and nymphs (43.2%). Among the 1500 ticks tested for *B. burgdorferi* s.l., 34.8% (n=522) were infected. Adult infection rate (39.6%) was higher than that of nymphs (25.5%). Infection load in real-time PCR ranged from 1 to 1.2 million spirochetes per tick. *B. afzelii* (39.7%), *B. burgdorferi* s.s. (12.1%), *B. garinii* (37.9%), *B. myamotoi* (3.6%), and *B. valaisiana* (23.8%) were recorded. *B. garinii* infected significantly less nymphs than adults whereas *B. afzelii* displayed the opposite trend. Survival rate of nymph and adult *I. ricinus* was significantly enhanced by infection by *B. burgdorferi* s.l. (χ^2; nymph: P=0.008, adult: P=0.021). In adults, a negative effect of infection on tick survival was observed when spirochete load overcame a threshold estimated at 160'000 spirochetes/tick, but not in nymphs. Moreover, ticks infected by *B. afzelii* survived better than other ticks (infected by other genospecies or not).

Discussion The results here indicate that infection by *B. burgdorferi* s.l., and more specifically infection by *B. afzelii*, confers survival advantages to *I. ricinus* under challenging thermo-hygrometric conditions.

Synthetic peptides for the diagnosis of human echinococcosis

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Introduction
The main source of antigen used in routine serodiagnosis of human echinococcosis is hydatid fluid of *Echinococcus granulosus* cysts collected from naturally infected intermediate hosts, e.g. sheep and cattle. Native and recombinant antigens are valuable reagents because they contain a great variety of epitopes. However, their disadvantage lies in substantial cross-reactivity that is routinely observed and thus in compromised test specificity. To avoid dependency on non-standardized native parasite material, effective strategies were sought for *in silico* selection of immunodominant epitopes that can be mimicked by chemically synthesized peptides.

Methods
We investigated three different approaches for the selection of parent proteins to design alpha-helical coiled-coil (CC) and intrinsically unstructured (IUR) peptides: i) known antigens [1]; ii) proteins identified by MS/MS from the diagnostically relevant 20-22kDa fraction of *E. multilocularis* vesicle fluid [2] and iii) conceptually translated EST libraries (Wellcome Trust Sanger Institute). The reactivity of the peptides with human sera was tested on a microarray platform. Promising candidates were further evaluated in ELISA format.

Key results
All peptides analyzed showed decreased sensitivity compared to native antigen, but the specificity was generally high, ranging from 90-96%. Selection approach i) produced the candidate with the highest sensitivity (57%). Peptides selected by approach ii) reached a sensitivity of 15% to 18%. Selection approach iii) identified a peptide deriving from an unknown protein reacting specifically with sera from *E. multilocularis* infected patients (27% sensitivity). These peptides can only be used in multiplexed assays.

Discussions / Conclusions
Each of the down-selected synthetic peptide does not present sufficient sensitivity when used alone, but on the grounds of their high specificity they have great potential for being combined and included into a multi-peptide assay. Future developments in serological diagnostics likely consists in highly pure and synthetic analytes in combination with new detection techniques compatible with multiplexing of diagnostic targets, e.g. Luminex microsphere based suspension arrays. We anticipate an increase in sensitivity and specificity in multiplexed testing. In particular gains in specificity are likely as individual cut-offs for each peptide could be maintained.

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Molecular approaches and functional analysis of potential drug target proteins in *Giardia lamblia*

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Abstract
Giardia lamblia is a common intestinal dwelling protozoan and causes diarrhea in humans and animals worldwide. To date, a limited number of drugs such as metronidazole (MTZ) and nitazoxanide (NTZ) are used for chemotherapy against human giardiasis. However, chemotherapeutical treatment of this disease has frequently been associated with recurrence of symptoms and formation of drug resistance, which was identified as one of the main reasons for treatment failure. Only little is known with respect to the mechanisms that lead to this important phenomenon.
In a study aimed at the examination of the mode of action of NTZ, we identified nitroreductase 1 (NR1) as a NTZ-binding protein in *G. lamblia* (WB C6) trophozoites. Furthermore, we overexpressed NR1 in *Giardia* trophozoites to elucidate its possible role in resistance formation. The overexpression was confirmed on the RNA level by RT-PCR and on the protein level by Western blotting. Now, transgenic parasites will be examinated regarding susceptibility / resistance to NTZ and MTZ.
Currently, we are investigating via differential 2D-gel electrophoresis proteins covalently binding either to either NTZ or metabolites emerging from treatment with this drug. Thereby, we found that drug treatment resulted in modification of proteins crucial for energy supply (e.g. ornithine carbamoyl transferase), detoxification of oxygen (e.g. NADH oxidase), and protein biosynthesis (e.g. elongation factor). We hypothesize that these protein modifications inactivate essential cellular functions and thus contribute to the parasitocidal effect of NTZ.

Arginine deiminase and ornithine carbamoyl transferase as pathogenicity factors of giardiasis

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Introduction

The intestinal protozoan G. lamblia is a major cause of human diarrhea worldwide, but there is only limited information on how G. lamblia causes disease, since the parasite is neither invasive, nor does it secrete any known toxin. Arginine deiminase (ADI) and ornithine carbamoyl transferase (OCT) are two giardial enzymes known to metabolize the amino acid arginine. Interestingly, G. lamblia releases these two enzymes upon interaction with human intestinal epithelial cells (IECs), which might lead into a depletion of host intestinal cells from arginine. Moreover, it has been shown that arginine depletion induces apoptosis in human cells. This suggests that ADI- and OCT-induced arginine-depletion could, via induction of IECs apoptosis, lead to leakage of the intestinal epithelium resulting in diarrhea.
Possible effects of arginine deprivation on IECs by giardial ADI and OCT are focused on in the present study.

Methods

Recombinant expression and purification of ADI and OCT in and from *G. lamblia.*
In vitro application of gADI and gOCT to human IECs (Caco2 and HCT-8):

a) Measurement of effects on cell numbers by MTT assay
b) Detection of apoptosis by DNA laddering
c) Detection of apoptosis by AnnexinV labeling

Key results

The two enzymes ADI and OCT were successfully expressed and purified in and from *G. lamblia* and were shown to be enzymatically active *in vitro*.

a) Upon *in vitro* application of the proteins to human IECs, cell numbers were reduced as determined by MTT assay. However, these preliminary results still need to be confirmed.
b) A protocol for DNA laddering upon induction of host cell apoptosis has been established. Analyses of DNA laddering in challenged cells are still ongoing.
c) AnnexinV labeling is being established and still ongoing.

Conclusion

This study investigates the first specific molecules of the host-parasite interface of giardiasis that play an important role in arginine depletion of host IECs. By metabolizing intestinal arginine, the parasite could affect IECs in a way that apoptosis is induced. First results from *in vitro* studies indicated that cell numbers were specifically decreased upon challenge with gADI and gOCT. Further analysis will reveal whether IEC apoptosis is indeed induced. Apoptosis of IEC would be an explanation for the diarrhea induced by *G. lamblia* infection.

First Case of *Anaplasma phagocytophilum* Seroconversion and Seroepidemiology in Northern Switzerland

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Introduction.
Human granulocytic anaplasmosis (HGA) has been suspected in Switzerland by serological evidence since 1995. More than 66 cases have been reported in Europe but none in Switzerland.

Material and methods.
Sera were collected from patients 2 months (PT2, n=203) after a tick bite with the control serum collected at the moment of the tick bite (PT0) and finally from patients presenting with Lyme borreliosis (LB, n=123). *Anaplasma phagocytophilum* IF slides (Focus Diagnostics, USA) were used to test sera at dilutions 1/32 and 1/64. Serum samples of the patient suspected with anaplasmosis were collected on Dec. 2007 and Feb. 2009. Thin blood smears collected on March 2009 were observed after Giemsa staining. One EDTA blood sample was analyzed by 16S gene PCR.

Results.
Dilution >1/32 was considered reactive for *A.phagocytophilum.* The patient's first serum was negative, the second collected 14 months later revealed a seroconversion with a titer at 1/2048. No other anterior serum was available. No morulae could be observed in granulocytes and PCR was negative. The patient showed no leucopenia, thrombocytopenia or any liver enzyme abnormality. Total seroprevalence in patients at PT2 was 7.4% and no seroconversion could be demonstrated for those 15/203 patients after testing PT0 sera. LB patients did not reveal a higher prevalence with 6,5% (n=8). No samples obtained titers >1/256.

Discussion and conclusion
Seroprevalence is comparable to results obtained in other European countries. With a prevalence around 1-4% of *A.phagocytophilum* in ticks, HGA should be actively considered and investigated. No typical clinical feature could be associated with this first seroconversion in Switzerland presenting evidence of an *A.phagocytophilum* infection

Scientific session 4:
Clinical Research & Public Health

Chairs: Ch. Lengeler, J. Lindgren

Etiology of fever in children from urban and rural Tanzania

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Introduction: Several studies have looked at the proportion of either malaria, pneumonia, diarrhea or bacteremia among fever cases in Africa but none of them at the overall spectrum of etiologies. We aimed at investigating the likely cause of fever episodes in children attending an outpatient clinic in both an urban and a rural
setting in Tanzania.

Methods: All consenting children aged 2 months - 10 years with a temperature >38°C were recruited. A detailed medical history and clinical examination were done to identify obvious foci of infection. A blood sample was taken to perform rapid tests for malaria and typhoid, blood culture and serological and molecular analyses. All had nasal/throat swabs taken for viral molecular investigation, urine when no obvious cause was found and stools when diarrhea was present. A chest X-ray was performed when IMCI criteria for clinical pneumonia were met. Each diagnosis was assigned a probability level (high, moderate, low) on the basis of pre-defined criteria.

Results: 1010 children were recruited, 510 in Dar es Salaam and 500 in Ifakara. Preliminary results on the causes of fever of high probability were: 50% acute respiratory infection (ARI) (31% URTI, 4% bronchiolitis, 12% non-documented pneumonia and 3% pneumonia documented by X-ray), 11% malaria, 9% diarrhea (3% rotavirus and 6% bacterial or unknown), 6% urine infection, 3% typhoid, 1% skin infection and 20% still unknown at this stage. 4% of the children had significant bacteremia, of which half were occult. 13% had more than one diagnosis (of high probability); 1% only had both malaria and pneumonia (documented or not). 104 children had a severe disease based on WHO criteria: 38% severe ARI, 36% severe malaria, 10% severe sepsis of unknown aetiology, 8% gastroenteritis with severe dehydration, 8% severe sepsis with another infection and 2% meningitis.

Discussion/Conclusion: These results provide for the first time an accurate picture of the causes of fever in African children. As expected, ARI contributed to the largest burden of disease, most of them being URTI. There was a sizeable proportion of fevers due to typhoid, as documented by the rapid test for most of them. Malaria confirmed to be less prevalent than generally thought. Results of molecular analyses and serologies will be presented and will provide further insight on the respective contribution of bacteria and viruses, a critical issue for appropriate management of fever and rational use of antibiotics.

Strongyloides stercoralis: methods of detection and efficacy of treatment in schoolchildren in Cambodia

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Introduction: Worldwide, about 30-100 millions people are infected with *Strongyloides stercoralis,* one of the most neglected soil-transmitted helminth (STH). Detailed information on the parasite is scare and diagnosis poses a problem. Our study aimed to compare two different diagnostic methods (Koga agar and Baermann technique) for *S. stercoralis* infection in multiple stool examinations and to assess the efficacy of ivermectin treatment.

Methods: We performed a cross-sectional study on *S. stercoralis* infection and STH in 458 children from four primary schools in semi-rural villages close to Phnom Penh by using different diagnostic procedures (Kato-Katz, Koga Agar and Baermann technique) on 3 stool samples. Infected children were treated with ivermectin (200mcg/kg PO, over 2 days) were reexamined 3 weeks after treatment.

Key results: Hookworms, *S. stercoralis*, *Trichuris trichiura* and small trematode eggs (STE) were frequently observed. 24.4% of children were infected with *S. stercoralis*. The sensitivity of Koga-Agar technique and Baermann method was 88.4% and 75.0%, respectively. The negative predictive value of both methods was 100%. The cumulative prevalence of *S. stercoralis* was considerably increased from 18.6% to 24.4 after analyzing 3 stool samples by either employed methods, which was much close to the modeled 'true' prevalence of 24.8%. The cure rate of ivermectin was 98.3%.

Discussion/Conclusions: *S. stercoralis* infection is highly prevalent among rural Cambodian schoolchildren. The sensitivity of Koga-Agar technique is higher than Baermann method (88.4% vs. 75.0%). In absence of a "gold standard test", the analyzing of multiple stool samples by different diagnostic methods is required. Ivermectin is highly efficacious against *S. stercoralis* infection.

Landscape Genetics Applied to Environmental Changes Impact on epidemiological systems in vector borne diseases: the Chagas disease model in Brazilian Amazon

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Introduction:
Landscape genetics is an emerging discipline whose aim is to describe impact of landscape structures on spatial and genetic structure of individuals. This approach is fundamentally used in population management and conservation biology because it can give relevant information on habitat connectivity. As part of the characterization of exposure scenarios of human populations at risk of emerging diseases and insect vectors, we applied this methodology to study the dispersion of the triatomine vectors of Chagas disease in Brazilian Amazon. Actually, there is a growing concern that the Amazon could become an area with an increasing risk of exposure to this parasitic disease, due to the proliferation of *Attalea* palms upon deforestation through human activities, this genus being the main forest biotope for triatomines. The integrated approach of population genetics and landscape ecology would be an excellent model for better understanding insects dispersion, parasite circulation and risk situation.

Method: The triatominae collection was realized by dissecting palms tree (*Attalea ssp*.) in three rural communities belonging to the Tapajos region, Pará State, Brazil. Palm trees were studied in the most heterogeneous and fragmented zones of the communities, being representative of the land cover modifications in the region. Genetic diversity of vectors was studied through 11 microsatellite loci and one mitochondrial gene (cytB), genotypic data were overlapped on a classified landscape map and landscape metrics measured.

Results: 752 insects (*Rhodnius robustus*) were collected from 148 palms from three main palm species (*Attalea speciosa, A. maripa, A.phalerata*). These palms were distributed in the three most representative landscape: forest, fallows and pastures.

Discussion: We aim to assess precise genetic relationships between individuals from a same palm tree and genetic flow between populations from different palms tree considering landscape discontinuities.

Agreement among various NSD diagnoses

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Introduction:
Neurasthenia Spectrum Disorders (NSDs) are conceptualized to cover the various diagnostic labels given to various disorders with essential core features of biomedically unexplained fatigue or weakness of six months duration causing sufficient distress to motivate help seeking and impaired work-capacity. These diagnoses are rarely used in Indian study setting. NSDs are debated universally over their validity, prevalence, etiology, clinical features, or management despite significant burden, medical pluralism, chronic course, and unsatisfactory outcome. Therefore, we decided to test the agreement among various NSD diagnoses.

Methods:
We recruited 352 patients from clinics of Psychiatry, Medicine, Dermatology, and Ayurved from Pune, India, meeting the essential criteria of core features. Diagnostic interviews were conducted for CFS and NT (ICD-10, CCMD-2, and DSM-IV). Interview instruments were translated and validated using appropriate methods. Data entry and analysis were done using Epi Info (6.04d) & BMDP. We compared the rates of diagnoses of Chronic Fatigue Syndrome (CFS, as defined by CDC), and Neurasthenia (NT by ICD-10, DSM-IV draft, and CCMD-2) among the NSD-patients.

Key Results:
Cochran's Q statistic yielded highly significant P values indicating different groups of patients identified by each category in each clinic. CFS was the least sensitive indentifying only 13.4% cases, followed by ICD-10 NT (24.1%), both being different across clinics. DSM and CCMD were similarly efficient across clinics, the latter having the most sensitivity (0.78). Only 8% patients met all four diagnoses. Two-way kappa statistic was just fair (0.4) for DSM and ICD, and poorer for all other comparisons. Agreement was the poorest (0.077) between CFS and CCMD-NT. Four-way concordance by Fleiss's method elucidated the high discordance furthermore.

Discussion and Conclusions:
The four categories have very poor concordance. Therefore, the operationally defined concept of clinical relevance devoid of etiological and cultural bias should be preferred. A cultural inquiry into subjective appraisal and illness behavior is essential

Tolerance and feasibility of Nifurtimox-Eflornithine Combination Therapy (NECT) for second stage *T. b. gambiense* HAT in Doruma, north-east DRC

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Introduction
Nifurtimox-Eflornithine Combination Therapy (NECT) has recently been approved in DRC for the treatment of second stage *T. b. gambiense* Human African Trypanosomiasis (HAT). We report here the result of the initial 6-month use of NECT in Doruma, north-east DRC.

Methods
Second stage HAT patients were hospitalized in Doruma Hospital and treated with NECT (Eflornithine 400mg/kg/d IV for 7 days with Nifurtimox 15mg/kg/d oral for 10 days), unless contra-indicated. Demographic and medical characteristics of the patients were recorded in Epitryps software. Clinical adverse-events were monitored during treatment and recorded on pharmacovigilance forms. We performed a retrospective analysis of data collected in Epitryps and the pharmacovigilance forms.

Key results
119 patients with second stage HAT were diagnosed and treated between December 2009 and June 2010 and 116 patients received NECT. Median age was 29 years (range: 1.5-70) and 33 patients (29%) were children ≤ 14 years old. Males were slightly predominant (55%). No patients died during treatment. Drug-related adverse events (AE) were reported in 100 patients (86%), including 92 patients (79%) with mild or moderate (grade 1-2) AE and 8 patients (7%) with severe or life-threatening (grade 3-4) AE (convulsions: n=3; psychosis: n=2; nausea/vomiting: n=3). Adverse-events were reported in 64% children, but none were severe. Gastrointestinal AE were the most frequently (62%) reported AE, including in children (51%). 114 (98%) patients completed NECT. The average duration of hospitalisation was 12.6 days.

Discussion/Conclusions
The use of NECT for treatment of second stage HAT was safe in a MSF supported treatment centre in north-east DRC. There were no deaths among 116 patients and severe AE were rare (7%). Tolerance in children was very good with no severe AE. Treatment of nausea and vomiting to prevent malabsorption of nifurtimox and good nursing care to prevent catheter-induced local or generalized infections are both crucial. Considering the shorter hospitalisation stay and the simplified scheme of eflornithine administration, NECT is more feasible than standard eflornithine therapy. The good safety profile and improved feasibility of NECT should facilitate the gradual switch from melarsoprol-based to eflornithine-based therapy for treatment of second stage HAT in all endemic countries.

Sero-epidemiological survey of gnathostomiasis in Lao PDR

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Human gnathostomiasis cases have been reported sporadically in Lao PDR since 1975, little is known about the disease in this country. Therefore, the sero-prevalence of gnathostomiasis and *Gnathostoma* species survey in Lao PDR were conducted in 2008 to 2009. One village each in the north, central and south regions of Lao PDR was selected as the study sites.

Overall, 125 (29.8%) of 420 sera from the randomly selected participants were sero-positive by immunoblot technique, with anti-*Gnathostoma* IgG antibody against the 24 kDa fraction. The sero-prevalence was high in the central (47.1%) and south (38.6%) regions, but very low (3.6%) in the north. Risk factor analyses revealed that the consumption of raw/undercooked fish was significantly associated with *Gnathostoma* sero-positivity (95% CI 1.05-17.05, P=0.042). The sero-positivity significantly increased with the age of the participants.

Several fish, swamp eels, and frogs collected from central and southern Lao were infected with *G. spinigerum* advanced 3rd-stage larvae. *Channa limbata* (Red-tailed snakehead fish) was identified as a natural second intermediate host of *G. spinigerum*. Eggs of *G. spinigerum* were found in dog feces collected in the south. Gnathostomiasis is endemic in central and southern Laos, so that preventive measures should be introduced for people living in these regions.

Key words: Sero-prevalence, Gnathostomiasis, *Gnathostoma spinigerum*, Food-borne nematode, Immunoblot, Lao PDR.

Scientific session 5:
Entomology
Chairs: A. Mathis, P. Müller

Microclimate and zoonotic cycle of tick-borne encephalitis virus in a risk area in Switzerland

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Introduction The distribution of tick-borne encephalitis virus (TBEv) appears to depend mainly on co-feeding transmission between infected *Ixodes ricinus* nymphs and uninfected larvae. To better understand the role of co-feeding ticks in the transmission of TBEv, we investigated tick infestation of rodents and the influence of microclimate on the seasonal evolution of questing ticks.

Methods A 3-year study was carried out in four sites in Canton Bern, including two TBE foci. Questing ticks and rodents were monthly collected and climatic data recorded. Ticks were screened for TBEv by real time RT PCR.

Results A decrease in questing tick density was observed in 2007, associated with low relative humidity and high temperatures in spring in all sites except one. The same year, the proportion of rodents carrying co-feeding ticks was also lower in sites where the decrease in questing tick density was observed whereas the proportion of infested hosts was similar over years. Globally TBEv was detected in 0.1% of questing ticks, and in 8.6% and 50% of ticks feeding on two rodents. TBEv was observed in 3/4 sites meaning that a new TBE focus was identified. In the site where no TBEv was detected, the percentage of rodents infested by at least one tick-stage was low, only 10% of rodents carried nymphs and the percentage of rodents with co-feeding ticks was the lowest (7-22%) versus (13-63%) in the 3 TBE foci.

Discussion A threshold in the proportion of rodents with co-feeding ticks seems to distinguish a TBE focus from a non-TBE focus. Since the enzootic cycle of TBEv may be locally affected by climate in spring - because of the influence of climate on co-feeding transmission- this cycle might be disrupted under dry and hot springs for consecutive years as predicted by Randolph (2001).

MALDI-TOF MS for characterization of *Culicoides* biting midges (Diptera: Ceratopogonidae)

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Identification of the tiny biting midges (*Culicoides* spp.) at present is primarily carried out using morphological features, particularly wing patterns, but is very difficult in many cases. For a number of species, several PCR-based tests have been developed. MALDI-TOF MS, which has become a routine method for identification of microorganisms in diagnostic laboratories, has recently shown promise in identification of metazoa. In a first step, we evaluated the potential of MALDI-TOF MS to consistently characterize laboratory-reared *Culicoides nubeculosus*. Sample preparation (ways of homogenization, matrix suspensions) was evaluated, and protein profiles were determined from a total of over 400 insects of both genders, of different age and duration of storage in 70% EtOH, of whole insects and body sections, of unfed and blood-fed females. Twenty-one reproducible potential biomarker masses were identified under different experimental treatments, including the homogenization of single insects in water allowing for additional DNA analysis. The biomarker masses were present independent of age, gender and different periods of storage of individuals in 70% EtOH. The presence of blood in females reduced the intensity of the MALDI-TOF pattern, necessitating the removal of the abdomen prior to analysis. Ongoing investigations reveal that MALDI-TOF MS which is a rapid, simple, reliable and cost-effective technique is suitable to identify species within the genus *Culicoides*.

An *in vitro* assay for testing mosquito repellents that employs a warm body and carbon dioxide as a behavioural activator *

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Introduction

Repellents are important in reducing contact between mosquitoes and humans, and appropriate assays are crucial to the development of more effective repellent products. We describe an *in vitro* behavioural assay for testing mosquito repellents applied in a dose-based manner to a warm body in test cages. The system was used to assess the sensitivity of *Anopheles gambiae* Giles to the insect repellent N,N-diethyl-3-methylbenzamide (DEET).

Methods

We recorded the number of landings by 4 - 6 day-old female *A. gambiae* in the test cages on a warm body (34°C) treated with increasing doses of DEET in absence and presence of a CO_2 pulse. The CO_2 pulse was applied to activate mosquitoes in the test cages.

Results

In absence of the CO_2 pulse the mosquitoes hardly responded to the warm body. Increasing the CO_2 level in the cage by 1000 ppm caused a 25-fold increase in the number of landings by mosquitoes on the warm body in 2 min tests. This mosquito activation allowed the measurement of a significant reduction in the number of landings to bite on the warm body with increasing doses of DEET (0.4 to 3.8 µg/cm^2). An asymptotic non linear model fitted to the repellency data in presence of CO_2 allowed estimation of the effective dose of DEET that reduced landings to bite by 50 % (ED_{50}) at 0.95 µg/cm^2 (5 nmol/cm^2) and the corresponding ED_{95} at 4.12 µg/cm^2 (21.5 nmol/cm^2).

Discussion/Conclusions

This *in vitro* bioassay has the advantage of permitting a fast throughput of test products under standardized conditions. This *in vitro* assay provides an alternative to human subjects for screenings designed to discover lead repellent products with as yet unknown human toxicological and dermatological profiles.

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Personal protection measure against ticks: *In vitro* and *in vivo* tests for the evaluation of active ingredients in tick repellent consumer products

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Introduction

The threat of transmission of Lyme borelliosis and FSME by *Ixodes ricinus* ticks in Switzerland has resulted in increasing number of tick repellents coming on to the Swiss market. These products aim to reduce contact between humans and ticks to prevent tick bites for the public and also for military and forestry personnel.

Methods

In the course of a mandate from the Federal Office of Public Health (BAG / OFSP) we used an *in vitro* assay on a warm glass plate and an *in vivo* assay on human legs to evaluate the repellent effects of the active ingredients of repellent sprays and lotions on the Swiss market.

Tick behaviours affected by repellents are falling off and walking down the glass plate or leg.
The *in vitro* assay is used for the initial evaluation of the dose range at which a product affects tick behaviour.

Results

Ticks show dose dependent responses to the compounds tested by falling off or walking down on both the warm glass plate and human leg. The most active compounds can be divided in to two groups depending on their effective dose range: DEET, EBAAP and Icaridin are active at doses at and above 0.01 mg/cm^2, whereas Lauric Acid, Capric Acid and Citriodiol® are active from a 10 times higher dose.

The strong similarity in tick behaviour responses to repellents on the warm glass plate and human leg assays prove the pertinence of the falling off and walking down behaviours for testing repellents.

Discussion/Conclusions

DEET, EBAAP and Icaridin are most effective as repellents against *I. ricinus* ticks. The dose levels found effective for these products serve as a guideline for the evaluation of consumer products containing these active ingredients.

The *in vitro* assay provides an alternative to human subjects for the evaluation of novel repellents with unknown human toxicity and dermatological effects.

Prevalence of zoonotic pathogens in questing *Ixodes ricinus* ticks in western Switzerland

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Introduction

Ixodes ricinus is known to transmit various pathogens, among them *Borrelia burgdorferi* sensu lato and the Tick-Borne Encephalitis virus, that are the agents of two diseases frequently diagnosed in European patients. The geographic distribution of these two microorganisms is subject of numerous investigations. In addition to these pathogens, *I. ricinus* may harbour *Rickettsia* spp., *Babesia* spp. and *Anaplasma phagocytophilum.* Information on their occurrence is scarce, especially in Switzerland. Therefore, a study was undertaken to evaluate the distribution of ticks infected by these pathogens as well as their prevalence.

Methods

In this study, more than 1000 nymphal and 300 adult *I. ricinus* ticks were collected from vegetation in 11 different sites located in the Western part of Switzerland in 2009 and 2010. Individual ticks were investigated for *Rickettsia* spp., *Babesia* spp. and *A. phagocytophilum* using polymerase chain reaction (PCR) followed by reverse line blot hybridization (RLB) for *Rickettsia* spp. (Jado et al. 2006) and *Babesia* spp. (Georges et al. 2001, Gigandet et al submitted), and by Real-time PCR for *A. phagocytophilum* (modified from Courtney et al. 2004).

Results

In this study, 13.1% of *I. ricinus* ticks were infected with at least one pathogen. Among the 3 pathogens, *R. helvetica* was the most frequently detected species and was recorded in ticks collected in all sites. *Babesia* spp., *A. phagocytophilum* and *R. monacensis* showed a more restricted geographic distribution and a lower prevalence, and the last pathogen was newly detected in this part of the country.

Conclusion

Some of these organisms are known to be pathogenic for humans. Therefore, physicians from these areas should be informed on their presence in ticks to make them aware on the possibility of clinical cases due to these tick-borne pathogens.

Identification of *Anopheles* mosquito species by molecular protein profiling

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Introduction
Vector control is the mainstay of malaria eradication programs. Successful vector control profoundly relies on accurate information on the target mosquito populations in order to choose the right intervention and to monitor its impact. As vectors show species-specific behaviour and ecology that may require different control measures, a key consideration is the assessment of mosquito species, abundance and distribution. An impediment to identify mosquito species is the existence of morphologically identical sibling species which, where available, may be distinguished by PCR diagnostics. DNA-based methods are, however, expensive, time-consuming and their development requires a priori DNA sequence information. In contrast, the recently described near-infrared spectroscopy for mosquito identification is less accurate.

Methods
To overcome the shortcomings of both approaches we evaluated an inexpensive molecular proteomics approach: matrix-assisted-laser-adsorption time-of-flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS is a well developed protein profiling tool for the identification of microorganisms but has rarely been used in insects. We measured MS spectra from specimens representing twelve Anopheles species including the five most important members of the A. gambiae complex.

Key results
Using multivariate statistics, MALDI-TOF MS accurately identified these species and single specimens could be assigned to their respective taxon of origin. The approach also reliably recognised patterns below taxonomic levels.

Discussion/Conclusion
While being exceptionally accurate and robust MALDI-TOF MS has several advantages over other typing methods, including simple sample preparation and short processing time. As the method does not require DNA sequence information about the mosquito, data can be reviewed at any later stage for diagnostic or functional patterns. This method has the potential to become an essential tool for many applications such as routine species identification, strain authentication, population genetics or even the detection of trait-specific markers including age or insecticide resistance.

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