# Differences in in vitro sensitivity and accumulation of anthelmintics in Trichuris suis and Oesophagostomum dentatum

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# Oral Presentations

## Track 1: Infectious Diseases

### 1.1 Non malarial febrile illness: challenges in epidemiology, diagnosis and treatment

#### O.1.1.001

**Why do health workers give antimalarials to RDT-negative patients? A qualitative study of factors affecting provider decision making at rural health facilities in Uganda**

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INTRODUCTION Large-scale use of malaria rapid diagnostic tests (RDTs) promises to improve fever case management and ensure the more rational use of antimalarials. However, evidence shows impact has been mixed. This study explored determinants of provider decision making to prescribe antimalarials following a negative RDT result.
METHODS A qualitative study was conducted in a rural district in western Uganda, 10 months after RDT introduction. To select health facilities for study, prescriptions for all patients with negative RDT results were audited from outpatient registers for a 2-month period at all facilities using RDTs ($n = 30$). Facilities were ranked by overall prescribing performance, defined as the proportion of patients with a negative result prescribed any antimalarial. 'Positive and negative deviant' facilities were selected; positive deviants ($n = 5$) were defined as <0.5% and negative deviants ($n = 7$) as >5%. Fifty-five fever cases were observed at the 12 facilities; all patients were interviewed. Twenty-two providers were interviewed. Analysis followed the framework approach.
RESULTS Eight thousand three hundred and sixty-eight RDT-negative patients were identified at the 30 facilities (prescription audit); 330 (3.9%) were prescribed an antimalarial. Of the 55 observed patients, 38 tested negative; one of these was prescribed an anti-malarial. Treatment decision making was influenced by providers' clinical knowledge and beliefs, capacity constraints, and interaction with the patient. Although providers believed in the accuracy of RDTs, a limited understanding of how the RDT works appeared to affect management of patients who already took artemisinin-based combination therapy. Patient assessment and other diagnostic practices were limited; providers lacked means for identifying alternative causes of fever. Provider perceptions of patient acceptance sometimes influenced treatment decisions.
CONCLUSIONS We found overall high provider adherence to test results, but that providers believed in clinical exceptions and sometimes felt they lacked alternatives. Clear communication on test functioning and better methods for assisting diagnostic decision making are needed.

### 1.2 Control of Neglected Zoonotic Diseases using a One Health approach

### Control of Neglected Zoonoses using a One Health approach

#### O.1.2.001

**A narrative of the neglected zoonotic diseases through the course of the World Health Assembly**

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Zoonotic diseases have been a priority of the World Health Organisation (WHO) since its inception in 1948. The zoonotic diseases of interest in this study are the eight Neglected Zoonotic Diseases (NZDs) that are the focus of the Integrated Control of Neglect Zoonoses (ICONZ) programme. Resolutions adopted at every World Health Assembly (WHA) held between 1948 and 2012 have been analysed to determine advocacy for the control of these diseases. Through the course of the WHA, 387 resolutions have been adopted that concern infectious diseases; of these only 20 resolutions directly concern the NZDs. Following the initial importance awarded to these diseases in the early stages of the WHO an historic analysis of the resolutions adopted for the NZDs shows how the level of advocacy in the global health community has reduced over the six decades of the WHO's existence. The nature of these diseases, such that they affect both human and animal health require inter-sectorial collaboration and renewed efforts to combat these diseases and reduce the neglect that is being seen in the advocacy for these diseases. The neglect of these diseases is particularly evident when the resolutions for brucellosis and rabies are examined; these diseases were both priority subjects of the WHO in its first decade, yet there have not been any specific resolutions for these diseases since this time.

### 1.4 Neglected Tropical Diseases

### 1.4.2 Dengue

#### O.1.4.2.001

**Innovative dengue prevention and control using permethrin-impregnated school uniforms: a mixed methods study on acceptability**

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There is no vaccine or specific antiviral treatment available for dengue currently. Control of the disease therefore focuses on effective vector control methods, which are limited. A novel and community-based approach is being investigated by the Dengue-Tools collaboration (www.denguetools.net) through a randomized controlled trial (RCT) assessing whether permethrin-impregnated school uniforms (ISUs) can reduce dengue incidence in



 

Chachoengsao Province, Thailand. This study provides a preliminary assessment of the acceptability of ISUs from the perspective of parents, school teachers and principals involved in the RCT. A triangulatory mixed methodological study was conducted. Class clustered randomised samples of students from four schools enrolled in the RCT were selected and their parents completed 321 self-administered questionnaires. Descriptive statistics and logistic regression were used for analysis of quantitative data. Focus group discussions and individual semi-structured interviews were conducted with parents, teachers, and principals. Qualitative data analysis involved content analysis with coding and thematic development. Knowledge and experience of dengue was extensive in study participants. Acceptability of ISUs was high. However, some participants indicated preference for awaiting trial results to determine acceptability. 87.3% (95% CI 82.9–90.8) of parents would allow their child to wear an ISU if provided for free. Some initial concerns regarding allergies to ISUs were ameliorated by experience with pre-trial permethrin treated wrist-bands. 59.9% (95% CI 53.7–65.9) of parents would incur additional costs for an ISU over a normal uniform. This was significantly associated with total monthly income of household and education level of respondent. Many thought ability to pay should be considered or ISUs should be provided free of charge. 62.5% (95% CI 56.6–68.1) of parents indicated they would be willing to recommend ISUs to other parents. At the early stages of the RCT, results were promising for acceptability of ISUs in the sub-population, should ISUs be shown effective in reducing dengue incidence.

## O.1.4.2.002

### Clinical management of dengue in 11 regional centres of excellence in seven countries across Asia and Latin America

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BACKGROUND Judicious intravenous (IV) fluid therapy is crucial for effective case management of dengue. We compare the fluid therapy of 1734 hospitalised dengue patients recruited to a prospective observational study in seven countries across Asia and Latin America.

METHODS All patients were followed daily using a single comprehensive case report form. Some of the participating clinical sites recruited children only.

RESULTS Crystalloids only were used for IV fluid therapy in 841 patients (48.5%); crystalloids and colloids in 94 patients (5.4%). For shock resuscitation crystalloids were used in the majority of the cases – in some sites also colloids (in 40–50% of shock cases, respectively). For rehydration crystalloids were used in 89.8% of the patients. The amount of fluids per 24 h period differed between the clinical sites – e.g. between 73.6 ml/kg in one site and 27.7 ml/kg in another site for rehydration fluids – or between 61.6 ml/kg and 15.6 ml/kg for maintenance fluids. Blood products were used in 68 patients (3.9%), of which 47 were severe patients. The remaining 21 patients received mostly platelets (50%), fresh-frozen plasma (FFP, 19.2%) and cryoprecipitate (15.4%). Between 0% and 3% of patients in most sites received blood products. However, blood products were given in one site to 11.3% of the patients, mainly FFP (34.5%) and platelets (25.9%).

CONCLUSIONS Considerable variation exists between clinical sites regarding the use of parenteral fluids and blood products for patients with dengue. Given the potential risks for fluid overload, work is needed to better define the optimal role of blood products and IV fluids for use in different age-groups and at different levels of severity.

## O.1.4.2.003

### The global spread of dengue virus serotypes: 1960–2012

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This review summarises the global spatial distribution of reported confirmed instances of each of the four dengue serotypes since 1960.

METHODS We have compiled the most extensive data set to-date by extracting information from published literature and case reports across a 53-year period. This resulted in a total of 2024 occurrences being mapped across the entire study period for DENV1, 1991 for DENV2, 1622 for DENV3, and 969 for DENV4. We describe the global spread of each serotype and the diversity of all four based upon sequential mapping through time.

RESULTS Each serotype spread from its focus in Asia in the 1960s to Africa and the Americas by the 1970s. The next two decades witnessed increases in the extents of all serotypes globally, although with poor documentation in Africa. Few areas had reported of all four serotypes until the 2000s, when Latin America and Asia saw dramatic increases in the number of serotypes having been reported.

CONCLUSIONS The maps and information provided here document the global spread of an increasingly important infectious disease, while highlighting the paucity of contemporary serotype information in Africa and underlining the importance of protection against all four serotypes in dengue vaccine development.

## O.1.4.2.004

### Dengue shock syndrome in children: clinical features and a prognostic model for profound shock

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INTRODUCTION There are no prospective reports describing the clinical features of dengue shock syndrome (DSS) in children, and no prognostic models for poor outcome.

MATERIAL AND METHODS We present a comprehensive description of DSS using data from two prospective studies (an observational study and a fluid intervention trial) of Vietnamese children with clinical DSS, subsequently confirmed by PCR or serology, managed on the paediatric ICU at the Hospital for Tropical Diseases, between 1999 and 2009. We developed a prognostic model for the primary outcome of 'profound DSS', here defined as death, major complications (severe bleeding, organ failure), or requirement for intensive treatment (two or more colloid boluses, inotropic support). We pre-defined candidate risk factors from among the features assessed at presentation. The prognostic model was based on logistic regression and is presented as a simple score-chart.

RESULTS The two studies included 1719 children with laboratory-confirmed DSS. Almost all patients had secondary dengue, and all four dengue virus serotypes were represented. Shock developed commonly between days 4 and 6 of illness. Clinical manifestations were generally consistent with empirical

descriptions of DSS, although 153 (9%) patients were still febrile and almost one third had no bleeding at presentation. Most patients responded well to standard fluid resuscitation and only eight children died. Prognostic model development was based on the 1210 children in the observational study, 225 (19%) of whom developed profound DSS. Younger age, earlier day of illness, higher temperature, higher haematocrit, faster pulse and worse hemodynamic status were independent risk factors for development of profound DSS. The final model had an AUC of 0.73 in temporal validation.
CONCLUSIONS This is the first prospective study to describe the clinical features of DSS and present a simple prediction score for severe outcome. These results should contribute to improvements in triage and management of children with DSS.

### O.1.4.2.005

**Dynamics of dengue virus serotype 4 intrahost genetic diversity in venous and skin-capillary blood**

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Dengue fever, an arthropod-borne disease caused by four serotypes of dengue virus (DENV-1,-2,-3,-4) is a global public health concern. Within the host DENV exists as a population of mutants with genetically related genomes. Previous studies suggested that the shape of the viral population evolves during the course of infection in human. However, this has only been explored in venous blood, whereas the viral population transmitted to the mosquito is the one contained in skin-capillaries. In the present study, the genetic diversity of DENV-4 was investigated in venous and skin-capillary blood samples serially collected from patients in acute phase of infection. The full envelope (E) gene was cloned and the sequences were analyzed. Compared to other serotypes, DENV-4 displayed the lowest intrahost genetic variability. When analyzing DENV-4 genetic diversity during the course of infection, we observed that the population evolved following three phases. The early-acute phase displayed the highest viral titres. During the middle-acute phase, the viral titers were high and the genetic diversity was low, as a suggested consequence of active replication of homogeneous DENV particles. In contrast, during the late-acute phase, the viremia was low and the genetic diversity was high, suggesting that the highly replicative homogeneous particles had been neutralized by the anti-DENV immune response. When comparing venous and skin-capillary blood samples, we found that DENV-4 genetic diversity was lower in skin-capillaries, suggesting that the viral population had been submitted to stronger selective constraints. Together with the observation that the virus titres were higher in skin-capillary blood, our results support the hypothesis of active replication of DENV in the vicinity of skin-capillaries, while DENV is no longer detectable in the venous bloodstream. Our study led to new hypothesis on the mechanisms contributing to DENV persistence in the skin that might extend the period of transmission to the mosquito.

### O.1.4.2.006

**Determining the main driver for the increase of epidemic dengue in Singapore**

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INTRODUCTION The frequency and magnitude of epidemic dengue have increased dramatically in the past 40 years as the viruses and the mosquito vectors have both expanded geographically in the tropical regions of the world. The reasons for the resurgence of dengue are complex. Often climate change has been blamed. Here we investigate weather and climate variables, population density and population increase, and incoming international arrivals as potential drivers for epidemic dengue in an industrialized city-state in South East Asia, Singapore, for the time period of 1974–2011.
METHODS We used a Poisson Regression Model, taking into account the annual number of dengue cases (DC) as dependent variable and population size (PS), Climatic Variables [max (Maxt), min (Mint), mean (Meant) temperature, Rain] premisses indexes (PI), Total number of visitors (Totvis) and visitors from Southeast Asia (SEAvis) as independent variables. As human population size can determine dengue activity by (i) providing new susceptible individuals that feed the epidemics; (ii) providing breeding places such that mosquitoes can increase in number; and (iii) providing sources of blood such that mosquitoes can reproduce and therefore increase in number, we also applied a basic Ross-Macdonald dynamic model, designed to describe the transmission of dengue in a given population.
RESULTS Population Size was signficantly associated with the observed number of dengue cases in the period analyzed. The Ross-Macdonald dynamical model resulted in a similar accuracy in predicting dengue cases compared to the Poisson Model. Both models showed that 1% increase in population size results in 5.5% increase in the number of dengue cases.
CONCLUSION The increase in population size in Singapore over the past decades appear to have been one of the main driving factor for epidemic dengue, and not climate change.

## 1.4.3 Mass drug administration and the control of neglected tropical diseases: learning from success, learning from failure

### O.1.4.3.001

**Uptake of preventive treatment for intestinal schistosomiasis among school children in Jinja District, Uganda: a cross sectional study**

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BACKGROUND In Uganda, the current national health sector strategic and investment plan underscores schistosomiasis as one of the diseases targeted for elimination by the year 2015. However, uptake of treatment among school children is unknown but suspected to be low. We estimated the uptake and predictors of preventive treatment with praziquantel.
METHODS In a cross sectional study carried out in Jinja district of Uganda, a random sample of 1010 children in 12 primary schools was questioned about their uptake of praziquantel, knowledge and perceptions about schistosomiasis,


 

support for taking preventive treatment and the dangers of taking praziquantel. The prevalence and mean intensity of infection with Schistosoma mansoni were determined.
RESULTS Self reported uptake of praziquantel at last mass treatment was 28.2% (95% confidence interval (CI): 22.9–33.6%). Overall prevalence and mean intensity of *S. mansoni* infection was 35% (95% CI: 25.4–37.9%) and 116.1 eggs per gram (epg) of stool (95% CI: 98.3–137.1) respectively. Uptake of praziquantel was more likely if a child was from a school with high prevalence of infection, had knowledge about schistosomiasis transmission and prevention and reported teachers' support to take praziquantel. Of the 285 children who took praziquantel, 142 (49.8%) developed side effects. Of the 725 children who did not take the drug, 522 (72.0%) reported fear of side effects as a major reason for non-uptake.
CONCLUSIONS Uptake of praziquantel in this population is very low. Fear of side effects of praziquantel, lack of knowledge about schistosomiasis transmission and prevention and lack of teacher support are some of the major factors associated with low uptake.
KEY WORDS Schistosomiasis, uptake of praziquantel, school children, Uganda

### O.1.4.3.002
**Factors influencing compliance to mass drug administration for control of lymphatic filariasis in Tanzania**

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Mass drug administration (MDA) has been adopted as a strategy to interrupt transmission of lymphatic filariasis and eventually eliminate the disease as a public health problem in endemic areas. To be effective, the MDA campaigns require careful planning, strong advocacy, mobilization and effective sensitization campaigns and high level of commitment from implementers at various levels of the programme. This study investigated the MDA process of the National Lymphatic Filariasis Elimination Programme (NLFEP) in Tanzania with specific focus on factors influencing drug coverage rates. The study aimed at documenting the MDA process and its associated drug coverage in urban and rural communities of Lindi and Morogoro Regions, before, during and shortly after the annual MDA activities. The study design comprised of qualitative and quantitative research components, including focus group discussions, in-depth interviews, observations and household-based cross-sectional questionnaire-based surveys. The research focused on: (i) Provider related factors in relation to NLFEP procedures for MDA implementation; and (ii) Population related factors, including socio-economic conditions, gender, age, area of residence and perceptions in relation to disease and programme information. Findings from the analysis of provider-related MDA activities and from the questionnaire based cross-sectional surveys will be presented and discussed. The findings will be used as a background for development of recommendations for improving programme performance and MDA delivery strategies in order to sustain a high treatment coverage and drug adherence in the target population.

### O.1.4.3.003
**HIV risk in women with female genital schistosomiasis (FGS)**

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INTRODUCTION Women with urogenital schistosomiasis have been found to have a three to four-fold higher odds ratio of having HIV. Histopathological studies have also shown increased vascularization and higher density of HIV target cells around schistosome ova in the genitals. The disease is characterised by so-called sandy patches causing genital mucosal inflammation and bleeding, especially on the cervix. Little is known about the biological mechanism behind the association of this waterborne parasite with HIV. In all areas endemic of schistosomiasis, heterosexual transmission is the most frequent mode of HIV transmission. The study set out to evaluate HIV target cells residing in the genital mucosa of women with FGS (Female Genital Schistosomiasis).
MATERIAL AND METHODS Participants were recruited from a larger school based study in KwaZulu-Natal, South Africa in a schistosomiasis and HIV endemic area. Blood and endocervical cytobrush samples were collected from 22 young women with genital sandy patches detected by colposcopy. The negative controls constituted 33 patients without genital lesions and without ova in urine microscopy. Flow cytometry was run in blood and cervical samples before treatment and 8 months after.
RESULTS FGS seems to increase the number and proportion of potential HIV target cells in blood and in the genital mucosa. The study indicates that treatment may influence this although the sample size is small.
CONCLUSIONS Further studies are needed to explore if treatment of FGS decreases the susceptibility to HIV.

## 1.4.5 Genital schistosomiasis

### O.1.4.5.001
**Examining the relationship between urogenital schistosomiasis and HIV infection**

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BACKGROUND Urogenital schistosomiasis, caused by infection with *Schistosoma haematobium*, causes substantial morbidity on the African continent. The infection has been suggested as an unrecognized risk factor for incident HIV infection. Urogenital schistosomiasis remains highly prevalent and underdiagnosed. This comprehensive literature review was undertaken to examine the evidence for a cause effect relationship between urogenital schistosomiasis and HIV/AIDS.

Methods We conducted a systematic search of academic and research literature. Plausible local and systemic mechanisms by which *S. haematobium* infection could increase the risk of HIV acquisition in both women and men area described. We detail the effects of *S. haematobium* infection on the progression and transmissibility of HIV in co-infected individuals. We summarize available evidence on the immunomodulatory effects of chronic schistosomiasis and the implications this might have for populations at high risk of both schistosomiasis and HIV.
Results Studies support the hypothesis that urogenital schistosomiasis in women and men constitutes a significant risk factor for HIV acquisition due both to local genital tract and global immunological effects. In those who become HIV-infected, schistosomal co-infection may accelerate HIV disease progression and facilitate viral transmission to sexual partners. Establishing effective prevention strategies using praziquantel is an important public health priority.
Conclusions Findings call attention to this pressing yet neglected public health issue and the potential added benefit of scaling up coverage of schistosomal treatment for populations in whom HIV infection is prevalent. The review supports discussions of urogenital schistosomiasis as a neglected yet urgent public health challenge.

### O.1.4.5.002

**Real-time schistosoma PCR in vaginal lavage and urine of high school girls in South Africa as an indicator of female genital schistosomiasis (FGS)**

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Brief Introduction It is hypothesised that early diagnosis and treatment of schistosomiasis among children and young girls may prevent or reduce Female Genital Schistosomiasis (FGS). In this study we evaluated detection and quantification of *Schistosoma* DNA in urine and vaginal lavage samples in young women as an indirect marker for FGS.
Materials and Methods Urines and cytology Pap smears were collected from 400 girls aged 16–21 years from rural high schools in KwaZulu Natal, South Africa. Vaginal lavage was performed by spraying 10 ml saline four times on the cervico-vaginal mucosal surfaces. Urines (20 ml) and Pap smears were examined microscopically for the presence of ova of *Schistosoma haematobium*. Quantification of *Schistosoma*-specific DNA by an ITS-based real-time PCR was performed on 200 ml aliquots of urine and vaginal lavage samples using an automated DNA isolation and PCR set-up.
Results *S. haematobium* eggs were seen in 71 (17.8%) of the urines. Schistosoma-specific DNA was amplified in 83 (20.8%) of the urines and 35 (8.8%) of vaginal lavages. In 27 (6.8%) participants real-time PCR was positive in both sample types. Ova were detected cytologically in 6 (1.5%) of the Pap smears, all showed detectable *Schistosoma* DNA in their lavage.
Conclusions The PCR results from the urine samples and those of the lavage samples compare well and suggest that genital schistosomiasis does exist in this young population. The lavage PCR showed almost six times more positive cases than Pap smear cytology. Further studies should be done to explore the clinical correlates of the findings.

### O.1.4.5.003

**Diagnosing female genital schistosomiasis**

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Introduction Female genital schistosomiasis (FGS) is a highly prevalent disease in some of the poorest areas of sub-Saharan Africa. Reliable and affordable diagnostics are unavailable in endemic areas. The lesions associated with FGS consist of changes in the genital mucosa that may be described as sandy patches, characterised by their yellow colour. The gold standard for diagnosing FGS is a biopsy from cervical lesions with direct microscopic inspection for ova. However, due to the transient risk of HIV transmission through the iatrogenic lesions, it is necessary to develop alternative methods for non-invasive, objective diagnosis of FGS that can be performed at the point of care without requiring advanced laboratory equipment or training.
Materials and Methods The image material was acquired in a study on female genital schistosomiasis in KwaZulu-Natal, South Africa. Healthy individuals served as controls. By analysing colour information in seven colour channels, we were able to differentiate sandy patches from surrounding mucosa. The validity was tested by running it on a random selection of images in which three clinicians had agreed on the diagnosis.
Results We found that the automated algorithm yielded a sensitivity of 80% and a specificity of 72% when compared to the clinical consensus group. The performance declined for decreasing image resolution and increasing levels of noise but was acceptable even for image qualities one would expect from less advanced cameras (5 Mpx, medium noise level).
Conclusion This is a novel method in which computerized image analysis can be used to identify genital schistosomiasis based on the lesions' distinct colour. By implementing the algorithm in cameras, it may serve as a useful diagnostic aid in endemic areas. Further research needs to be done on acceptability and patient security.

## 1.4.7 Diagnosis of helminth infections

### O.1.4.7.001

**Mini-FLOTAC, Kato-Katz and McMaster: three methods, one goal**

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Background Copro-parasitological diagnosis is still a challenge in the management of helminth infections both at individual and community levels in resource-limited settings. The aim of our study was to compare three quantitative diagnostic techniques: Kato-Katz thick smear, McMaster technique and mini-FLOTAC, an innovative method based on floatation of helminths eggs with two different solutions (FS2 saturated saline and FS7 zinc sulphate). The study was carried out in a parasitology laboratory in Oran, Northern Argentina.
Results Out of 193 schoolchildren examined, 40% resulted positive for any helminth infection by any method; the most prevalent was *Hymenolepis nana* (23%) followed by *Ascaris lumbricoides* (17%) and a third group of less prevalent


 

helminths such as Enterobius vermicularis, Trichuris trichiura and hookworm (11% all together). The mini-FLOTAC resulted the most sensitive technique: 93% for *H. nana*, 91% for *A. lumbricoides* and 95% for other helminths. Comparing Kato-Katz and McMaster methods, the former was more sensitive for *A. lumbricoides* (84% vs. 53%) and for other helminths (48% vs. 43%) except for *H. nana* (49% vs. 62%). Between the two flotation solutions, FS2 was more sensitive for *H. nana* (98% vs. 81%) and for other helminths (85% vs. 80%), whereas FS7 resulted more sensitive for *A. lumbricoides* (97% vs. 69%). As for egg counts, mini-FLOTAC reported higher eggs per gram of feces (EPG) for *H. nana* (906 vs. 457 with McMaster and 111 with Kato-Katz) and for *A. lumbricoides* (1820 vs. 1314 with Kato-Katz and 995 with McMaster); between the two solutions, FS2 detected the highest EPG for both helminth infections (906 vs. 568 for *H. nana* and 1177 vs. 643 for *A. lumbricoides*) although the difference was not statistically significant.
CONCLUSIONS Mini-FLOTAC is a promising technique for helminth diagnosis, more sensitive than the Kato-Katz and the McMaster.

## O.1.4.7.002

### Comparing different endemic settings on the distribution of soil transmitted helminths by using stool-based multiplex real-time PCR

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INTRODUCTION The diagnostic procedure commonly used to monitor infections with Soil Transmitted Helminths (STH) is detection and quantification of parasite eggs or larvae by stool microscopy. Despite being a relatively simple procedure, it is highly observer dependent and influenced by the laboratory procedures used. This lack of standardization obstructs comparison of data collected at different study sites. Recently, real-time PCRs were developed and validated for the detection and quantification of a wide range of parasitic infections which proved to be sensitive and highly specific. Here we analysed data collected from two different endemic regions.
METHODS Population based cross-sectional surveys on prevalence and intensity of STH were performed in Beira, Mozambique ($N$ = 303) and Flores Island, Indonesia ($N$ = 1087). In Mozambique five different microscopy procedures were used on each collected stool sample, while logistic conditions allowed only limited microscopy detection in Indonesia. Samples were analysed in a centralized laboratory using similar multiplex real-time PCR procedures, detecting and quantifying parasite specific DNA of *Ascaris lumbricoides*, Necator americanus, Ancylostoma duodenale and *Strongyloides stercoralis*.
RESULTS Based on overall microscopy, parasite species-specific prevalence in Mozambique ranged from 35% to 53%. PCR-based detection rates were higher than each individual microscopy technique, but comparable to the combined data of all microscopy procedures. In Indonesia real-time PCR detected substantially more positive cases than microscopy, in particular for hookworm (17% vs. 73%). Multivariate analysis of the Indonesia data showed real-time PCR detected STH infections to be associated with behavioural risk factors.
CONCLUSION Multiplex real-time PCR was found to be a highly sensitive, reliable and relatively simple high throughput procedure to monitor STH infections in cross sectional surveys. The use of multiplex real-time PCR for the detection and quantification of helminth DNA in stool samples allows for improved comparison of the epidemiology of these infections between different geographical regions.

## O.1.4.7.003

### Comparison of diagnostic accuracy of five serodiagnostic tests for strongyloidiasis

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INTRODUCTION The diagnosis of *Strongyloides stercoralis* infection is hampered by the suboptimal sensitivity of faecal-based diagnostic methods. Serology is felt to be more sensitive, but less specific. Assessing the accuracy of serologic methods may be difficult, precisely because of the lack of sensitivity of faecal-based gold standard.
MATERIALS AND METHODS A retrospective study on 399 anonymized serum samples from a cryopreserved biobank (CTD) assessed five different serologic tests, three at CTD (in-house IFAT and the commercially available Bordier ELISA and IVD ELISA) and two at NIAID-NIH, Bethesda, based on the recombinant Ss-NIE1 antigen (in-house NIE-ELISA and NIE-LIPS [Luciferase Immunoprecipitation System]). Endpoints were accuracy measures of the five tests using faecal results as primary reference standard, but also using a composite reference standard – defining infected as patients with positive stool OR at least three positive serologic tests and uninfected as patients with negative stool AND at least three negative serologic test.
RESULTS Based on these criteria, there were 130 infected subjects and 269 uninfected controls, of which 65 had another (non-Strongyloides) parasitic infection. The most sensitive test was the IFAT, with a sensitivity 94.6% (91.2–96.9), followed by IVD ELISA with sensitivity 92.3% (87.7–96.9). The most specific test was the NIE-LIPS, with specificity 99.6% (98.9–100), followed by IVD ELISA with specificity 97.4% (95.5–99.3). LIPS appeared to cross-react with only 1/65 (1.5%) specimens from subjects with other parasitic infections. ROC analysis indicate that LIPS, IFAT and the two commercial ELISAs reach virtual 100% specificity at a cut off level that maintains ≥70% sensitivity (ROC for IVD is reported as an example in Figure).
CONCLUSION NIE-LIPS is the most accurate serologic test for the diagnosis of *S. stercoralis* infection. IFAT and the two commercial ELISAs are sufficiently accurate, above a given cut off, for diagnosis, prevalence studies and inclusion in clinical trials.

## 1.4.8 Progress towards alternative anhelminthic drugs

### O.1.4.8.001

**A meta-analysis of comparative and non-comparative studies of praziquantel (PZQ) for treating intestinal and urinary schistosomiasis**

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INTRODUCTION Despite two Cochrane systematic reviews of treatments for *S. haematobium* (Sh) and *S. mansoni* (Sm) infections, it is not easy to derive comprehensive information on the efficacy of praziquantel in urinary and intestinal schistosomiasis.
METHODS A systematic search (September 2012) identified 273 studies, of which 92 were eligible (comparative or non-comparative clinical trials of single-agent praziquantel), and 58 (42 were comparative, 16 non-comparative) analysed (efficacy end-point within 8 weeks post-treatment). Efficacy was assessed as cure rate (CR) and egg reduction rate (ERR).
RESULTS Studies were conducted between 1979 and 2012 at 82 sites in 24 countries (Africa: 74 sites, 21 countries) and enrolled around 18 500 patients (praziquantel = 14 100, control/placebo = 4400). Thirty-four studies were on Sm (10 700 patients, 57%), 20 on Sh (5100 or 29%), four on *S. japonicum*, four on mixed infections. The largest population was schoolchildren: 10 800 (57%; 4800 infected with Sh). Praziquantel dose ranged 10–60 mg/kg. 40 mg/kg was given to 10 400 (74%), 60 mg/kg to 1700 (14%). Efficacy was assessed within 4 weeks in 8300 (45%) and between 5 and 8 weeks in 10 200 (55%). PZQ efficacy (CR, ERR) varied with dose and species. By random-effect meta-analysis of controlled trials, the CR with PZQ 40 mg/kg of Sm was higher than 20 mg/kg (RR = 0.65, 95% CI 0.59–0.72, $P = 0.001$) and 30 mg/kg (RR = 0.86, 95% CI 0.77–0.95, $P = 0.004$), and not different from higher doses (50 mg/kg, $P = 0.544$; 60 mg/kg, $P = 0.477$). For Sh, no difference was detected between 40 mg/kg and other doses. No difference was detected in ERR between groups but using multivariate analysis with random effects, the ERR increased with PZQ dose in patients treated for Sh ($P = 0.027$).

Conclusions are based on a large database. 40 mg/kg (the WHO-recommended dose) is effective at all ages in all forms of schistosomiasis.

### O.1.4.8.002

**Efficacy of a single oral dose of 8 mg Moxidectin vs. 150 μg/kg ivermectin in onchocerca volvulus infection: results of a randomized, double-blind single dose phase 3 study in areas without mass treatment with ivermectin in Liberia, Ghana and DRC**

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INTRODUCTION Around 100 million people live in onchocerciasis-endemic areas. Control of onchocerciasis as a public health problem in Africa relies on annual ivermectin mass treatment. Recently, elimination of transmission was added to control programme objectives. Moxidectin, a veterinary anthelmintic, is evaluated for its potential use for mass treatment to shorten time to elimination relative to ivermectin and/or allow elimination where ivermectin does not.
METHODS Moxidectin or ivermectin were given to 978 and 494, respectively, males and females ≥12 years with ≥10 microfilaria/mg skin (mf/mg).
RESULTS For participants with 12 month evaluation (96.6% moxidectin treated and 97.2% ivermectin treated), mean ± SD mf/mg pre-treatment, 1, 6, 12 and 18 months after treatment were 39.1 ± 30.9, 0.1 ± 0.5, 0.0 ± 0.1, 1.3 ± 3.0 and 4.3 ± 8.8 in moxidectin treated and 41.1 ± 31.5, 2.3 ± 7.0, 3.6 ± 6.1, 9.9 ± 12.9 and 15.3 ± 18.3 in ivermectin treated, prevalence of microfilaria positive skin snips was 100%, 6.7%, 5.0%, 35.3% and 61.7%, respectively, among moxidectin treated and 100%, 51.7%, 81.9%, 91.3% and 96.6% among ivermectin treated. At all time points, moxidectin was significantly more effective than ivermectin ($P < 0.0001$).
CONCLUSIONS Since relative efficacy in a study does not allow conclusions on relative impact on disease control, the results were compared with a transmission model. The average reduction in mf levels during 1 year post treatment (moxidectin 95.0 ± 1.9, ivermectin 84.4 ± 15.4, $P < 0.0001$) evaluated relative to the transmission model by Duerr et al. suggests that the higher efficacy of moxidectin would increase the threshold biting rate and allow elimination of onchocerciasis with mass treatment alone in areas where the model suggests this is not possible with ivermectin.

## 1.4.9 Trypanosomiasis and leishmaniasis

### O.1.4.9.001

**Chagas disease – can a threshold for bug infestation rate exist?**

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BRIEF INTRODUCTION The control of infestation of *T. dimidiata*, one of major vectors of Chagas disease, is a primary strategy for elimination of the disease. However, the degree to which domestic infestation of the bug needs to be reduced is not known. This study is aimed at assessing the existence of a threshold for the domestic infestation rate of *T. dimidiata*, below which transmission becomes unlikely.
MATERIALS AND METHODS A census was conducted in 59 Chagas disease endemic communities in El Salvador and Honduras to examine the existence of the threshold for the domestic infestation rate of *T. dimidiata*. Both entomological and serological tests were conducted targeting all 4083 households and 6324 children between 6 months and 15 years of age in the communities. Domestic infestation rate of *T. dimidiata* assessed using the One-Person-Hour method for bug investigation; sero-prevalence of Chagas disease estimated using ELISA IgG as serological tests for *T. cruzi*.
RESULTS AND CONCLUSION The overall domestic infestation rate of *Triatoma dimidiata* and sero-prevalence among children were 12.9% and 0.49%, respectively. Communities with a domestic infestation rate at 8% or less consistently showed a

  

sero-prevalence of 0% (Chi square test: $P < 0.001$). There was a variety in sero-prevalence of the communities with a domestic infestation rate above 8%. An infestation rate of 8% could serve as the threshold below which transmission would become unlikely. Implementation of an 8% threshold should lead to a 21% reduction in spraying-related costs. A crude estimate further indicates a savings of US$ 13.0 per household by avoiding unnecessary spraying and its accompanied interventions.

**Table 1** Relationship between *T. dimidiata* domestic infestation rate threshold 8% and sero prevalence

| | Sero-prevalence in communities | | |
|---|---|---|---|
| | =0% | >0% | Total |
| Number of communities | | | |
| *T. dimidiata* domestic infestation rate of communities | | | |
| 0–8% | 29 (49.2%) | 0 (0%) | 29 (49.2%) |
| >8% | 13 (22.0%) | 17 (28.8%) | 30 (50.8%) |
| Total | 42 (71.2%) | 17 (28.8%) | 59 (100%) |
| Chi square test | | | $P < 0.001$ |
| Number of children between 6 months and 15 years of age | | | |
| *T. dimidiata* domestic infestation rate of communities | | | |
| 0–8% | 3250 (51.4%) | 0 (0%) | 3250 (51.4%) |
| >8% | 1371 (21.7%) | 1703 (26.9%) | 3074 (48.6%) |
| Total | 4621 (73.1%) | 1703 (26.9%) | 6324 (100%) |
| Chi square test | | | $P < 0.001$ |

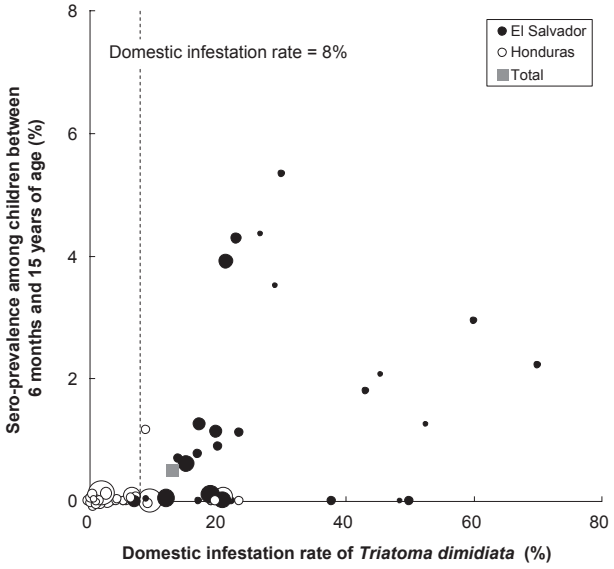


**Figure 1** Relationship between domestic infestation rate and sero-prevalence.

## O.1.4.9.002

### Detection of human-infective trypanosomes in clinical samples from domestic animals and tsetse flies from Zambia's tsetse-infested regions using LAMP

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Trypanosomiasis is one of the re-emerging debilitating diseases of livestock and humans in sub-Saharan Africa, caused by various trypanosome species transmitted by tsetse flies. Several domestic and wild animals act as reservoirs of various trypanosome species. In Zambia, Human African trypanosomiasis (HAT) is endemic in Luangwa valley and there are increasing unpublished cases being reported in HAT old foci. Current available parasitological methods used for trypanosomiasis diagnosis are either less sensitive, unable to accurately identify species and may sometimes be laborious. Loop-mediated isothermal amplification (LAMP) is a novel strategy which amplifies DNA with high sensitivity and rapidity under isothermal conditions. In the present study, the performance of trypanosome-species-specific LAMP including the human serum resistance-associated gene (SRA)-LAMP assay were evaluated using clinical specimens obtained from domestic animals and tsetse flies from Zambia's Luangwa and Zambezi river valleys. Various animal-infective trypanosome species including Trypanosoma congolense, *T. brucei brucei* and *T. vivax* were detected in both parasitaemic and aparasitaemic individuals by trypanosome-species-specific LAMP. Importantly, we detected the human-infective *T. b. rhodesiense* by SRA-LAMP (confirmed by SRA-PCR) in cattle (1%) and dogs (5.3%), suggesting their possible role in HAT epidemiology. The prevalence of *T. b. rhodesiense* in tsetse vectors was 3.3% (19/576), exclusively involving Glossina morsitans morsitans. LAMP is thus a potential simple and cost-effective tool for diagnosis of trypanosomes and other infections in resource-limited endemic regions. Satelite analysis further revealed a similar genotype of *T. b. rhodesiense* circulating among tsetse vectors and mammalian hosts.

## O.1.4.9.003

### NECT feld phase IIIb trial: final effectiveness in adults and children results

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BRIEF INTRODUCTION *Trypanosoma brucei* (T.b.) gambiense Human African trypanosomiasis (HAT; sleeping sickness) is a fatal disease. Until 2009, available treatments for 2nd stage HAT were complicated to use (eflornithine monotherapy), expensive or toxic and insufficiently effective in certain areas (melarsoprol). Nifurtimox-eflornithine combination therapy (NECT) recently demonstrated good safety and efficacy in a randomised controlled trial (RCT) and was added to the World Health Organisation (WHO) List of Essential Medicines (EML) for adults in 2009. Documenting its effectiveness at 24 months after treatment under field conditions will further support its use.

MATERIAL AND METHODS A multicentre, open label, single arm, phase IIIb study of the use of NECT for 2nd stage T.b. gambiense HAT. All patients who could take oral medication were treated with NECT. Children and pregnant women were included for the first time. Follow-up visits were done every 6 until 24 months after end of treatment.
RESULTS Between May 2009 and May 2010, 630 patients were included; 100 of them were children below 12 years of age, 13 were pregnant women and 34 were breastfeeding women. Only 15 patients were completely lost to follow-up. Final outcome shows 94% effectiveness (577/614), with similar results in adults (484/515) and children below 12 years of age (93/99). Final safety results brought 28 deaths (11 related) and a total of 67 serious adverse events (SAE). Children and pregnant women had less adverse effects or mortality than other adults. Ninety-two percent of patients showed at least one adverse event.
CONCLUSIONS The use of NECT for treatment of second stage HAT is safe and effective in field settings, both in adults and in children. NECT was submitted for inclusion on the WHO Model List of Essential Medicines for children in December 2012.

## O.1.4.9.004

### Phase IIb clinical trial with posaconazol and benznidazol for chronic Chagas disease

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INTRODUCTION The current treatment against Chagas disease is bounded to benznidazole and nifurtimox. The cure rates in chronic phase of the disease are around 8–40%. Posaconazole has revealed a powerful antitrypanocidal activity in murine model. Its antiparasitic mechanism and activity and its safety profile in humans, makes posaconazole a promising treatment against Chagas disease.
MATERIAL AND METHODS A prospective, randomized, clinical trial was designed to assess the efficacy of posaconazole in adult patients with chronic infection. Three different arms were considered: posaconazole 400 mg/12 h for 2 months (High dose posaconazole-HDP); posaconazole 100 mg/12 h for 2 months (Low Dose posaconazole -LDP) and benznidazole 150 mg/12 h for 2 months (BNZ). Twenty-six patients were included in each arm. The primary endpoint was antiparasitic activity measured by a real time PCR (rtPCR) in blood sample at 10 month after treatment. Biochemical tests and EKG were performed at days 7, 14, 28, 45 and 60 during treatment, and at month 2, 4, 6 and 10 after ending treatment. Parasite load measured by a rtPCR was also determined at each visit. A pharmacokinetic curve at 14th day was performed to those patients receiving posaconazole.
RESULTS A total of 78 patients were enrolled. Four patients withdrawn the treatment due to severe cutaneous adverse reactions in the BNZ arm. None of the patients had alterations of the EKG. In the Per Protocol analysis 90.5% and 80% of patients receiving LDP and HDP respectively had positive rtPCR during follow up period compared with 5.9% in the BNZ arm. Posaconazole concentrations in serum were similar with those previously published.
CONCLUSIONS Patients treated with posaconazol (regardless of the dose) have greater proportion of treatment failures compared to those receiving BNZ. Posaconazole treatment was safe and better tolerated than BNZ. During treatment period, posaconazole has shown an anti-Trypanosoma activity.

## O.1.4.9.005

### Malaria – visceral leishmaniasis co-infections in East Africa

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Due to geographic overlap of malaria and visceral leishmaniasis (VL), co-infections may exist but have been poorly investigated. Two studies have been undertaken to further gather data on malaria-VL co-infections in East Africa. To describe prevalence, features and risk factors for VL-malaria co-infections, a case-control analysis was conducted on data collected at Amudat Hospital, Uganda by Médecins sans Frontières. A second retrospective study was performed using medical records of VL patients admitted to Tabarakallah and Gedarif Teaching Hospitals (Gedarif State) and Al'Azaza kala-azar Clinic (Sennar State), Sudan. In Uganda, of 2414 patients with confirmed VL, 450 (19%) were positively diagnosed with concomitant malaria. Most co-infected patients were males, residing in Kenya (69%). Young age was identified as a risk factor for concurrent VL and malaria, particularly the age groups 0–4 and 5–9 years. Mild and moderate anemia negatively correlated with the co-morbidity. VL patients harboring skin infections were nearly three times less likely to have the co-infection. Anorexia was slightly more frequent among co-infected patients. The in-hospital case-fatality rate did not significantly differ between cases and controls, being 2.7% and 3.1% respectively. In Sudan, the prevalence of malaria co-infection among VL surveyed patients ranged from 3.8% to 60.8%. Co-infected patients presented at hospital with deteriorated clinical pictures; emaciation, jaundice and moderate anemia were found to be positively associated with the co-infection. Severity of splenomegaly and, to a lesser extent, hepatomegaly (OR: 0.52; 95% CI: 0.27–1.01) appeared to be reduced by concomitant VL and malaria. The in-hospital case-fatality rates did not significantly differ between co- and mono-infected patients. Co-infections do occur in endemic VL areas with malaria transmission, indicating that routine screening of VL patients living in malaria endemic-areas and close monitoring of co-infected patients should be implemented.

## O.1.4.9.006

### Drug delivery by tattooing to treat cutaneous leishmaniasis

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INTRODUCTION Leishmaniasis is a vector-borne disease caused by obligate intra-macrophage protozoa of the Leishmania species. Leishmaniasis can cause different clinical syndromes, including cutaneous leishmaniasis (CL), in which the patient presents with one or several ulcer(s) or nodule(s) in the skin


 

resulting from the infection of macrophages located in the dermis. Although there is a huge clinical need, no good treatment is available for CL at the moment. This project investigates the hypothesis that a tattoo device can target intradermal drug delivery against CL parasites.
MATERIAL AND METHODS The selected drug is oleylphosphocholine, an alkylphosphocholine in the same family as miltefosine which can be formulated as nanoliposomes that can be delivered by tattooing. *In vitro* efficacy of the formulated drug was first established in cultured macrophages. Subsequently, mice infected with *L. major* or *L. mexicana* and presenting skin lesions were treated using a 10-day regimen. *In vivo* efficacy of the tattoo-mediated drug delivery was evaluated at the clinical and parasitological levels and compared to other more classical modes of administration.
RESULTS Cultured Leishmania-infected macrophages can engulf drug-containing nanoliposomes resulting in a direct dose-dependent killing of intracellular parasites. On infected mice with cutaneous lesions, a 10-day tattoo-mediated treatment resulted in rapid clinical recovery with complete regression of skin lesions. Parasite counts and histopathological examination confirmed efficacy of the treatment. No damage was observed to the skin architecture beyond those normally seen from the tattooing procedure.
CONCLUSION This study establishes proof-of-concept that localized tattoo-mediated delivery of an antileishmanial drug is effective in treating CL. The low amount of drug required and the high efficacy of this treatment method may have a positive impact on CL patient management. Future studies will measure drug levels in the skin layers over time following tattoo-mediated therapy in view of transposing the results to clinical studies.

## 1.4.10 Soil-transmitted helmintiasis

### O.1.4.10.001

**The impact of a health education intervention on soil-transmitted helminth infections in grade 5 schoolchildren of the Peruvian Amazon: a cluster-randomized controlled trial**

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INTRODUCTION Soil-transmitted helminth (STH) infections are an important health problem in developing countries. To control these infections, the World Health Organization recommends school-based deworming programs with a health hygiene education component. The objective of this study was to rigorously investigate the effectiveness of a health education intervention incorporated into a school-based deworming program in Grade 5 schoolchildren in Bélen, Peru.
MATERIALS AND METHODS An open-label pair-matched cluster-randomized controlled trial was conducted in Grade 5 classrooms of 18 primary schools (nine intervention and nine control) in the Peruvian Amazon. The intervention consisted of: (i) a 1 h in-class activity and 30-min refresher activities every 2 weeks over 4 months; and (ii) a half-day workshop for teachers and principals. Al lschoolchildren were administered single-dose albendazole following baseline STH assessment. At 4 months follow-up, STH-related knowledge, STH-related risk behaviours and STH infection were re-assessed.
RESULTS From April 21 to October 20, 2010, 518 and 571 schoolchildren, from intervention and control schools, respectively, participated in this study. At follow-up, intervention children scored significantly higher (44% higher) on all aspects of a test of STH-related knowledge compared to control children. Furthermore, intervention children had lower odds of bathing in the contaminated river (aOR = 0.72; 95% CI: 0.48–1.06) and of drinking water directly (aOR = 0.58; 95% CI: 0.44–0.76) compared to control children. The intensity of Ascaris infection at follow-up was statistically significantly lower in children in intervention schools compared to children in control schools (aIRR = 0.42; 95% CI = 0.21–0.85). No significant changes in hookworm or Trichuris intensity were observed.
CONCLUSIONS The school-based health hygiene education intervention effectively increased STH knowledge and reduced infection. The benefits of school-based deworming programs are likely to be enhanced when a sustained health hygiene education intervention is integrated into school curricula.

### O.1.4.10.002

**Improvement of serodiagnosis of human strongyloidiasis**

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Cross reactivity with other parasitic infections, particularly filariasis has always been one of the major drawbacks in enzyme-linked immunosorbent assay (ELISA) towards diagnosis of strongyloidiasis. In this study, specific in-house ELISAs were developed for detection of IgG, IgG4 and IgE antibodies against *Strongyloides stercoralis*. A commercial ELISA (IVD Research, USA) was also used, and the sensitivities and specificities of the four assays were determined. Sensitivity of the assays were assessed using 26 serum samples from patients with *S. stercoralis* infection while the specificities were evaluated using 55 serum samples from patients with other infection and healthy individuals. Sensitivities of the IgG4, IgG, IgE and IgG (IVD) assays were 76.9%, 84.6%, 7.7% and 84.6%, respectively, while the specificities were 92.7%, 81.8%, 100% and 83.6%, respectively. If filariasis samples were excluded, the specificities of the IgG4-ELISA and both IgG-ELISAs increased to 100% and 98%, respectively. A significant positive correlation was observed between IgG- and IgG4-ELISAs ($r = 0.4828$; $P = 0.0125$). IgG- and IgG- (IVD) ELISAs ($r = 0.309$) were positively correlated, but was not significant ($P = 0.124$). Meanwhile there was no correlation between IgG4- and IgG- (IVD) ELISAs ($r = 0.0042$; $P = 0.8294$). Sera from brugian filariasis patients showed weak, positive correlation between the titres of antifilarial IgG4 and the optical densities of anti-Strongyloides IgG4-ELISA ($r = 0.4544$, $P = 0.0294$). In conclusion, the detection of both anti-Strongyloides IgG4 and IgG antibodies could improve the serodiagnosis of human strongyloidiasis. Furthermore, patients from lymphatic filariasis endemic areas who are serologically diagnosed with strongyloidiasis should also be tested for filariasis.

## O.1.4.10.003

### Stool examination of 410 asymptomatic school children in a highland village of Madagascar

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INTRODUCTION Parasitic and bacterial enteric pathogens in endemic countries such as Madagascar are often present in individuals without acute symptoms. Such 'asymptomatic carriers' may suffer from long-term effects and play an important role as disease reservoirs and sources of infection.
METHODS We collected stool samples of 410 children aged 4–18 years from a primary and secondary school in a Madagascan highland village in the Ambositra region. DNA was isolated and three multiplex PCR panels targeting bacterial (Salmonella spp., Yersinia spp., Shigella spp. EIEC, *Campylobacter jejuni*) and protozooic (*Entamoeba histolytica*, *Giardia lamblia*, Cryptosporidium spp., Cyclospora spp.) pathogens and Schistosoma spp. were applied.
RESULTS Of the bacterial PCRs, 22% were positive for *Campylobacter jejuni* and 14% for Shigella EIEC spp. No evidence for Salmonella and Yersinia was found. The protozoaen parasite *Cyclospora cayetanenisis* was found in 16% and *Giardia lamblia* in 51%. Only one individual was positive for Cryptosporidium spp. The PCR for *Schistosoma mansoni* was positive in more than 77% of all children, 70% in girls and 86% in boys (chi2-test $P < 0.0001$). While infestation proportions for *Giardia lamblia* steadily decreased with age (from 66% in 4–8 year olds to 29.5% in 14–18 year olds), for *Schistosoma mansoni* it increased from 61% in 4–8 year olds to 89% in 14–18 year olds.
CONCLUSION A positive PCR does not prove active infections with gastrointestinal, but (recent) past or current infestations. Nevertheless, the high proportion of Schistosoma PCR positives, indicates that repeated praziquantel mass treatment and improved water sanitation are required to lower the burden of Bilharziosis in the Madagascan highlands. The relative high proportion of children positive for Campylobacter spp. and *Cyclospora cayetanensis* are probably due to contact with livestock.

## O.1.4.10.004

### Helminth infections and micronutrients in schoolchildren: a systematic review and meta-analysis

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INTRODUCTION Helminth infections and micronutrient deficiencies are both highly prevalent in developing countries. Neither condition typically causes overt disease but they do lead to indirect morbidity and impaired physical and cognitive development.
OBJECTIVE We aimed to systematically review current evidence on the relationship of helminth infections with micronutrient status in schoolchildren worldwide.
MATERIAL AND METHODS We included both observational studies and RCTs. We used random effects meta-analysis (i) to estimate cross-sectional associations between helminths and micronutrient status; (ii) to estimate anthelminthic treatment effects on micronutrient status, and (iii) to estimate effects of micronutrient supplementation on helminth (re)infection.
RESULTS Meta-analyses of observational studies showed a significant association between helminth infections and serum retinol [SMD (standardized mean difference) −0.30 (−0.48; −0.13)] but not serum ferritin [SMD 0.00 (−0.7; 0.7)]. Conversely, meta-analyses of anthelminthic RCT studies did show a positive effect on ferritin [SMD 0.14 (0.08; 0.20)] but not on retinol [SMD 0.04 (−0.06; 0.14)]. We did not find enough studies to pool data on other micronutrients besides ferritin and retinol. When evaluating helminth (re)infection rates in micronutrient supplementation studies, only multi-micronutrient interventions showed a modest protective effect [OR 0.77 (0.61; 0.97)].
CONCLUSIONS We found significant associations between helminth infections and micronutrient status in schoolchildren. Our results showed distinct associations with either serum retinol or serum ferritin. More evidence is needed to further unravel the interrelationship between helminth infections and micronutrient status.

## O.1.4.10.005

### Malnutrition and the critical growth window in an STH-endemic area of Peru: worm infections a barrier to achieving child-related MDGs?

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INTRODUCTION Children under 2 years of age are in the most critical window for growth and development. As mobility increases, this time period also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical environments. The association between malnutrition and worm infection, however, has been understudied in this vulnerable age group.
MATERIALS AND METHODS A double-blind, randomized, placebo-controlled trial of a deworming intervention is currently being conducted in 12-month old children in Iquitos, an STH-endemic area of the Peruvian Amazon. Baseline enrolment data was collected between September 2011 and June 2012. Anthropometric measurements were taken, including length and weight. Stool specimens were collected from all participants, with half of the specimens (i.e. from those receiving active mebendazole treatment) being analyzed immediately with the Kato-Katz technique. The association between malnutrition and STH infection was analyzed using logistic regression.
RESULTS A total of 1760 children were enrolled into the trial. Baseline data of all participants showed a prevalence of stunting, underweight and wasting of 24.2%, 8.7% and 2.3%, respectively. Of the 880 participants with analyzed stool specimens, the prevalence of any STH infection was 14.6%. The distribution by parasite species was 11.5% for Ascaris, 4.5% for Trichuris and 0.6% for hookworm. A significant association was observed between malnutrition (stunting) and infection with at least one STH species (aOR = 1.68; 95% CI: 1.06, 2.66).
CONCLUSIONS Although previously thought to be negligible, the high STH prevalence by 12 months of age and the associated link with malnutrition is of concern. Follow-up of these children at 18 and 24 months of age is nearing completion. This data will provide increased insight into the longer-term effects and causality between STH infection and malnutrition, such that appropriate interventions can be targeted to this vulnerable age group and improvements in child-related MDGs can be achieved.

  

### O.1.4.10.006

**Comparison of individual and pooled stool samples for the assessment of soil-transmitted helminth infection intensity and drug efficacy**

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BACKGROUND In veterinary parasitology samples are often pooled for a rapid assessment of infection intensity and drug efficacy. Currently, studies evaluating this strategy in large-scale drug administration programs to control human soil transmitted helminths (STHs; *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm), are absent. Therefore, we developed and evaluated a pooling strategy to assess intensity of STH infections and drug efficacy.
METHODS AND MATERIALS Stool samples from 840 children attending 14 primary schools in Jimma, Ethiopia were pooled (pool sizes of 10, 20, and 60) to evaluate the infection intensity of STHs. In addition, the efficacy of a single dose of mebendazole (500 mg) in terms of fecal egg count reduction (FECR; synonym of egg reduction rate) was evaluated in 600 children from two of these schools. Individual and pooled samples were examined with the McMaster egg counting method.
RESULTS For each of the three STHs, we found a significant positive correlation between mean fecal egg counts (FECs) of individual stool samples and FEC of pooled stool samples, ranging from 0.62 to 0.98. Only for *A. lumbricoides* was any significant difference in mean FEC of the individual and pooled samples found. For this STH species, pools of 60 samples resulted in significantly higher FECs. FECR for the different number of samples pooled was comparable in all pool sizes, except for hookworm. For this parasite, pools of 10 and 60 samples provided significantly higher FECR results.
CONCLUSIONS This study highlights that pooling stool samples holds promise as a strategy for rapidly assessing infection intensity and efficacy of administered drugs in programs to control human STHs. However, further research is required to determine when and how pooling of stool samples can be cost-effectively applied along a control program, and to verify whether this approach is also applicable to other NTDs.

### O.1.4.10.007

**Prevalence of strongyloidiasis in North East Italy – preliminary data from an observational serosurvey**

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INTRODUCTION Due to its characteristic autoinfection cycle *Strongyloides stercoralis* (*S. stercoralis*), when not treated, can persist in the host's organism for years. Autochthonous cases of *S. stercoralis* infection have been documented in the past in Italy, but knowledge of the current epidemiological situation is limited. The aim of this survey is to collect data on the prevalence of strongyloidiasis in people (Italians and immigrants) currently living in North East Italy.
MATERIALS AND METHODS The study started on February the 4th 2013, and is still ongoing. Outpatients (Italians born before 1951 and immigrants older than 18 years) were enrolled at the clinical laboratories of seven hospitals. During each of the randomly-selected enrolment weeks, we collected blood samples from 10 patients with ('cases') and 10 patients without ('controls') eosinophilia (defined as eosinophil count >500 cells/$\mu$l). Sera were screened with a commercial ELISA kit (IVD); positive samples were re-tested with an in-house IFAT technique. Possible discordant cases were tested with a third method (Bordier ELISA).
RESULTS From February the 4th to May the 2nd 2013, we had analyzed 388 serum samples. Twenty two patients resulted positive at the screening test, and 19 of them were confirmed positive (around 5% of screened patients). Mean value of eosinophil count in the 19 patients was 780 cells/$\mu$l. Only one positive patient was in the 'control' group (with an eosinophil count of 420 cells/$\mu$l). Mean age of the 10 positive Italians was 76 years, while mean age of the nine immigrants was 35 ears.
CONCLUSIONS The preliminary results of this serosurvey show that *S. stercoralis* infection is present in the observed areas. In the autochthonous population, strongyloidiasis it is still an issue in the elderly.

## 1.5 Malaria

## 1.5.1 Malaria immunology

### O.1.5.1.001

**Exposure dependency of antibody levels and memory B-cell frequencies in *Plasmodium falciparum*-exposed Ghanaian women**

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Protective immunity to *Plasmodium falciparum* malaria acquired after natural exposure is mediated by IgG antibodies targeting the asexual blood stages of the parasites. Antibodies with specificity for members of the PfEMP1 proteins expressed on the surface of infected erythrocytes appear to be of particular importance in this respect. The transience of antibody responses and the slow rate of acquisition of protective immunity probably reflect the extensive clonal antigenic variation and allelic polymorphism of key antigens such as PfEMP1. However, it is likely that other immune-evasive mechanisms are also involved, such as interference with formation and maintenance of immunological memory. In this study, we measure PfEMP1-specific antibody levels and memory B-cell frequencies in a cohort of *P. falciparum*-exposed non-pregnant Ghanaian women. The antigens used are VAR2CSA, where expression is restricted to parasites infecting the placenta, and two commonly recognized PfEMP1 proteins (HB3var06 and IT4var60) implicated in rosetting and not restricted to pregnancy. This approach allows us to compare directly and in the same individuals the immune responses specific for a clinically important parasite antigen (VAR2CSA) where expression is confined to well-defined periods (pregnancy) with responses specific for comparable antigens that are continuously/regularly expressed in a manner independent of pregnancy (HB3var06 and IT4var60). We will present data on B-cell phenotypes, antigen-specific IgG levels, and antigen-specific memory B-cell levels, as well as their associations with each other, with number of previous pregnancies, and with time since last pregnancy.

### O.1.5.1.003

**Erythrocyte polymorphisms associated with protection against *P. vivax* malaria in Papua New Guinea children**

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INTRODUCTION Malaria parasites have long coexisted with hominid ancestors: *P. falciparum* might had infected humans before the out-of-Africa migration and followed the human expansion throughout the tropics. This long-term interaction may have driven the co-evolution of the host-parasite and left evolutionary foot-prints in both human and parasite genomes. According to the 'malaria hypothesis', the high frequencies of deleterious mutations of some human populations are probably due to the strong selective pressure of malaria. A remarkable range of polymorphisms have been associated with protection against malaria. Populations of the South West Pacific, a co-endemic region for all four human malaria parasite species, are highly diverse and exhibit a range of unique red blood cell polymorphisms, with geographical patterns paralleling malaria endemicity.
METHODS AND RESULTS In an earlier study we showed that South East Asian Ovalocytosis (SAO) (caused by band 3 deletion SLC4A1Δ27) was associated with a reduction reduction in risk of clinical *P. vivax* episodes and infections and revealed the potential for a strong protection against severe *P. vivax* malaria. Here we present results on the association of Gerbich blood group variants (caused by a deletion in exon3 of the glycophorin C gene (GYPC) with risk of *P. vivax* and *P. falciparum* in PNG children. In a cohort of children 1–3 years, GYPC homozygote genotype is associated with strong protection against *P. vivax* malaria and the strength of protection increases with parasitemia. In addition, we will present the data from two further cohorts of 3–21 months and 1–4 years from PNG.
CONCLUSION This strongly suggest that *P. vivax* malaria may have contributed to shaping the unique host genetic adaptations to malaria in Asian and Pacific populations.

### O.1.5.1.004

**Maintenance of immunity to severe malaria: a retrospective clinical study in travelers with imported malaria in Sweden**

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INTRODUCTION Immunity against severe malaria develops faster than against mild and uncomplicated forms of the infection. In addition, this immunity is believed to be life-long under repeated exposure, reflected by the low incidence of severe malaria in older children and adults living in malaria endemic areas. Adult immigrants from malaria endemic areas are well recognised to be at reduced risk of severe malaria. To what extent immunity against severe malaria wanes in the absence of re-exposure remains to be established.
MATERIAL AND METHODS In the present study, we assessed the clinical presentation in adult travelers diagnosed with *P. falciparum* malaria in Sweden 1995–2012. We performed a detailed review of medical records and demographic data from 800 patients diagnosed with malaria at Karolinska University Hospital in Stockholm. Severity of disease was assessed with regards to WHO criteria and admission to the intensive care unit.
RESULTS AND CONCLUSION Patients originating in a malaria endemic country in Sub-Saharan Africa had an overall lower risk of severe malaria than patients of non-endemic origin (mainly Sweden). Interestingly, the number of years of residency in Sweden among the African immigrants affected their risk of severe malaria. The results suggest that although immunity to severe malaria is maintained for several years without re-exposure, this protection is not life-long. The findings have implication on the management of malaria in travelers and contribute to the understanding of maintenance of immunity against malaria.

## 1.5.2 Malaria and iron deficiency

### O.1.5.2.001

**The impact of intermittent parasite clearance on malaria, anaemia, and cognition in schoolchildren: new evidence from an area of highly seasonal transmission**

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Malaria control has usually focussed on pregnant women and children under 5, in whom the risk of malaria-related mortality is greatest. Yet some studies have shown that older school-age children could also benefit from malaria control, with potential gains for both health and education. Mali recently introduced universal coverage of nets, with community-wide distributions starting in 2011. Whilst successful in achieving high levels of coverage, 80% of schoolchildren remained infected at the end of the malaria transmission season in November 2011. This calls for additional control measures in this age group. One potential supplementary strategy is intermittent parasite clearance in schools (in which a treatment dose is given irrespective of infection status) with the aim to improve educational performance by reducing malaria-related anaemia and improving cognitive function. This approach is particularly suited to areas of seasonal transmission where a single annual treatment can be given in school at the end of the transmission season.
METHODS A cluster-randomized controlled trial was conducted in 80 primary schools in Sikasso, Mali where the majority of schoolchildren already slept under insecticide-treated nets. Children in intervention schools received a single treatment dose at the end of November 2011; administered in school by teachers over three consecutive days.
RESULTS Parasite clearance was associated with dramatic reductions in malaria parasitaemia and gametocyte carriage at follow-up in February 2012 in intervention compared to control schools. This effect was sustained until May 2012, the beginning of the next transmission season. Malaria parasite clearance was also associated with a significant decrease in anaemia (OR = 0.56, 95% CI 0.39–0.78, $P = 0.001$), and increase in sustained attention ($P < 0.001$). The findings will be discussed in relation to the growing body of evidence on the impact of asymptomatic malaria infection on cognitive performance in schoolchildren, and control strategies in areas of highly seasonal transmission.


 

## 1.5.3 Experimental malaria

### O.1.5.3.001
**Intranasal administration of artesunate and erythropoietin to treat cerebral malaria**

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INTRODUCTION Despite treatment with anti-malarial drugs, patients die from cerebral malaria and too many are discharged with neurological deficits. In the long term, 1/4 of survivors have neurological and cognitive impairments years after exposure. Currently, treatment of malaria is based mainly on drugs such as artesunate. Erythropoietin has been shown to play an important role in limiting cell injury and apoptosis. Human trials with rhEPO have demonstrated tissue protective activities in multi-system diseases requiring intensive care support. We first demonstrated the protective effect of rhEPO in murine cerebral malaria (Kaiser 2006, Bienvenu 2008). We also demonstrated that high doses Erythropoietin were safe for children suffering severe cerebral malaria (Picot 2009). Use as adjunctive treatment associated with anti-malarial drug, Epo could help to decrease the early mortality rate of severe malaria.
MATERIAL AND METHODS Experimental malaria was induced in mice infected with *P. berghei* ANKA. Animal experiments were conducted in agreement with ethical and general rules for animal protection. Drugs were used at serial dilutions and administered either I.P. (control groups), using intranasal spray device, or dropwise after anesthesia. Mice were monitored for signs of CM, including body weight, hematocrit, ataxia, paralysis, deviation of the head, convulsions, and coma. Parasitemia were determined using Giemsa-stained thin blood films.
RESULTS AND CONCLUSION Intranasal administration of artesunate was highly efficient to decrease blood parasitemia in *P. berghei* infected mice. The cure rate using 40 mg/kg b.w; was 100% for two successive experiments (60 mice). Same efficacy was obtained when the artesunate dose was deacreased. This is the first evidence that artesunate and erythropoietin treatment could be administered via intranasal route.

### O.1.5.3.002
**Pre-clinical model of a genetically arrested experimental malaria vaccine**

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INTRODUCTION Upon an infected mosquito bite, malaria parasite sporozoites specifically invade the liver, where they develop into thousands of merozoites that initiate the clinical blood stage infection. This pre-erythrocytic infection is the target of cell-based experimental malaria vaccines. Repeated immunizations with high doses of live, metabolically active sporozoites can induce protracted protection against Plasmodium re-infection. In our recent work using a murine malaria infection model we showed that parasites lacking the Plasmodium-specific apicoplast protein PALM arrest prior to liver merozoite release and elicit sterile protection with two immunization doses only.
MATERIALS AND METHODS Here, we tested the duration of protection elicited by infections with late liver stage-arrested parasites. Immunized mice were challenged >1 year later and parasite liver loads quantified. We also profiled antigen-specific CD8+ T cell responses.
RESULTS We found that two doses of 10 000 late liver stage arrested Pbpalm(-) sporozoites induce unprecedented, long-lasting protection against challenge with normal sporozoites. Partial protection extended beyond 1 year after the last immunization. In aging mice, surface markers of CD8+ effector memory cells were uniformly detectable, including in non-immunized control animals. In contrast, intracellular cytokine staining of Plasmodium peptide-stimulated hepatic CD8+ T cells revealed elevated levels of interferon gamma in vaccinated mice.
CONCLUSIONS Our data indicate that antigen-specific effector T cells persist in the target organ even in aging mice. Moreover, quantification of antigen-specific immune effector cells correlates with protracted protection in an experimental murine malaria vaccine model. Together, our findings show that a defined developmental arrest prior to liver stage merozoite release has the capacity to elicit potent immune responses against re-infection.

### O.1.5.3.003
**Extensive damage to the endothelial glycocalyx in terminal murine cerebral malaria**

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INTRODUCTION Malaria parasites invade erythrocytes and to avoid splenic clearance interact with and bind to the lumen of blood vessels in the microcirculation. This interaction activates the endothelium and in concert with immune processes leads to several downstream events including patchy endothelial apoptosis. This is particularly prominent in cerebral malaria (CM). We addressed the effects on the endothelial glycocalyx in murine malaria models. The glycocalyx is a complex matrix of proteoglycans and glycoproteins with importance for upkeeping endothelial function and homeostasis and it is the layer with which infected erythrocytes makes its initial contact upon endothelial binding.
MATERIALS AND METHODS C57BL/6 mice were infected with *Plasmodium berghei* ANKA causing murine CM or with *P. chabaudi* AS causing uncomplicated malaria and compared with uninfected mice. Tissue was either processed for transmission electron microscopy, focussed ion beam-scanning electron microscopy or for glycan composition by lectin blotting. Plasma was analysed with ELISA and dot blots for relevant markers.
RESULTS The cerebral, endothelial glycocalyx was either completely lost or collapsed in terminally ill CM mice. Less pronounced but similar changes were found in uncomplicated malaria, whereas uninfected mice had normal glycocalyx. The changes were seen in the vasculature of the brain, an organ with pronounced pathology during CM, but not in the heart, an organ with little pathology. The loss of endothelial glycocalyx was associated with increased levels of glycocalyx components in the plasma.
CONCLUSIONS The demonstration of gross endothelial glycocalyx damage as a component of CM pathology warrants further studies of its contribution to the pathogenesis of severe malaria and its role in the interaction with parasitized erythrocytes and parasite variant surface antigens.

## 1.5.4 Challenges in anti-malarial drug resistance

### O.1.5.4.001

**Active, prospective monitoring of antimalarial drug safety**

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INTRODUCTION Today, malaria-endemic countries can chose between antimalarials with comparable efficacy, but different safety profiles. However, while efficacy assessment is common, safety is often overlooked; safety liabilities of drugs are known in clinical trials, far less in real-life.
METHODS A 9-year active safety survey in peripheral dispensaries in rural Senegal, implemented in parallel with the staggered introduction of test-and-treat practice. A questionnaire was designed and staff trained to record occurrence and severity of signs/symptoms before, during, and after treatment (28-day follow-up). Lab tests were in >10%.
RESULTS Three thousand seven hundred and eight (52% of 7144 artesunate-amodiaquine treatments given during 2000–2011) were enrolled. Monitoring safety was overall satisfactory but varied in numbers and quality. The sample size was adequate to characterize known reactions. The availability of a denominator allows quantifying frequencies. Collecting the occurrence and intensity pre- versus post-treatment reduces background noise and improves quality and specificity of the signal. Collecting information on dose and weight allows verifying adequacy of dosing with different formulations (loose, co-blister, fixed).
CONCLUSIONS Data have benchmarking value. Active monitoring of safety according to the principles of cohort event monitoring (CEM) is feasible in peripheral dispensaries and informative for known toxicities (parmacovigilance more suited for signal generation). Linking health system facilities and communities minimized losses to follow-up; attention must be paid to signal-to-noise (signs/symptoms that could be caused by malaria or the medication) and spectrum composition (the relevant settings, users and providers); mechanisms should be set in place for ensuring consistency, training and quality control, and for the sharing and use of information. More studies are needed to assess if such system is sustainable, where, and what would make it cost-effective.

### O.1.5.4.002

**Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine in *Plasmodium falciparum* malaria patients in Central Vietnam**

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INTRODUCTION Reduced susceptibility of *Plasmodium falciparum* towards artemisinins including confirmed resistant cases, have been reported from the Thai-Cambodian, and Thai-Myanmar borders. In Vietnam, delayed parasite clearance times (>72 h) have been increasingly reported from Southern provinces bordering Cambodia. This study aimed to assess potential delays in *P. falciparum* parasite clearance in Quang Nam province, situated at the Eastern coast of Central Vietnam.
MATERIALS AND METHODS The study was designed as a 42-day follow-up study to assess the efficacy of Dihydroartemisinin-piperaquine (DHA-PQ) for the treatment of uncomplicated *P. falciparum* malaria. Patients were treated with an age depended dosing scheme of DHA-PQ according to national treatment guidelines, and monitored 12-hourly until complete parasite clearance. The primary endpoint was parasite clearance, and secondary endpoints included treatment efficacy at day 42, as well as the IC50 and IC90 of both dihydroartemisinin (DHA) and piperaquine (PQ).
RESULTS A total of 95 patients were enrolled between September and December 2012. The median parasite clearance half-life was 6.2 h (IQR: 2.59; 11.9) and the median parasite clearance time was 62 h (IQR: 48; 83). A total of 26 (29.2%) patients showed parasites on day 3. No early treatment failure occurred, and late parasitological failures occurred in two patients on day 35 and 42, respectively. PCR unadjusted adequate clinical and parasitological response at day 42 was 97.7%. PCR adjusted efficacy as well as *in vitro* sensitivity results will be presented.
CONCLUSION Clearance times and rates were higher compared to previous studies in southern Vietnam, and similar to those reported from Western Cambodia. Our results suggest that artemisinin resistance may have reached the eastern coast of Central Vietnam, requiring confirmation by mono-therapy trials. Containment efforts should be reinforced in the Greater Mekong subregion.

### O.1.5.4.003

**The effect of dosing regimens on the antimalarial efficacy of dihydroartemisinin piperaquine in patients diagnosed with uncomplicated malaria: a pooled analysis of individual patient data**

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INTRODUCTION The fixed dose antimalarial dihydroartemisinin-piperaquine (DHA-PIP) is deployed increasingly in malaria endemic countries as the Artemisinin-based Combination Therapy (ACT) of choice.
METHODS To assess whether dosing strategies are associated with treatment efficacy, individual patient data were shared with the World-Wide Antimalarial Network (WWRN) and collated using standardised methodology. Cox's regression model with shared frailty across study sites was used to assess the risk factors associated with treatment failure.
RESULTS Data from 26 clinical efficacy studies conducted in 7072 patients with uncomplicated malaria between 2002 and 2011 (Africa: 4009, Asia: 2807, and S. America: 256) representing 77% of the targeted published data were included in the analyses. Overall, the drug was very efficacious; PCR-adjusted Kaplan–Meier (K-M) estimates of efficacy were 98.8% (95% CI: 98.5–99%) at day 28, 97.7% (95% CI: 97.3–98.1%) at day 42 and 97.2% (95% CI: 96.7–97.7%) at day 63, but K-M estimates dropped by day 63 to 94.4% (92.6–96.2%) in children 1–5 years. Children from 1 to 5 years received a significantly lower dose of piperaquine (53.3 mg/kg, IQR: 45.7–64.0) compared to those 5–12 years old (57.1 mg/kg, IQR: 51.2–65.5, $P < 0.001$) and those <1 year (60 mg/kg, IQR: 53.3–67.6, $P < 0.001$). The mg/kg drug dosage of PIP was a significant risk factor for PCR confirmed treatment failure in multivariate analysis after adjusting for known confounders (AHR: 0.97, 95% CI: 0.96–0.99, $P = 0.002$). Increasing the target total dose of piperaquine in this subgroup from 54 to 59 mg/kg would result in at least 95% of children being cured by day 42.


 

CONCLUSIONS Although the overall treatment efficacy of DP is excellent, treatment failure is associated with suboptimal dosing, particularly in young children. Further dose optimization may be warranted to retard the onset and spread of drug resistance and prolong the useful therapeutic lifespan of this valuable ACT.

### O.1.5.4.004
**Statistical estimation of malaria genotype frequencies: a Bayesian approach**

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Methods that enable the characterisation of *P. falciparum* genotype frequencies may have important implications for public health, including the surveillance of antimalarial drug resistance. Resistance is most often a complex trait, which requires that several different genetic markers occur in a single parasite (a particular haplotype or genotype). Estimating *P. falciparum* genotype frequencies is nontrivial, since individuals are often infected with multiple parasite clones, which mask the alignment of individual markers in the genomes of the constituent clones, rendering the genotypes ambiguous. Without making biased assumptions, direct counting methods of genotype frequency cannot incorporate data from multiclonal blood isolates, resulting in the loss of valuable data. A Bayesian statistical model, which uses a Metropolis-Hasting Markov chain Monte Carlo sampling scheme, has been developed to estimate the *P. falciparum* genotype frequencies from data collected in the field. It utilises all the data, including those from multiclonal infections and those that have partially missing information, for example, due to genotyping failures. The utility of the model was demonstrated using previously published data from a study of markers in two *P. falciparum* genes, dihydrofolate reductase and dihydropteroate synthase, mediators of resistance to sulfadoxine-pyrimethamine, which were collected as part of a drug efficacy trial in Uganda. Four biologically relevant genotypes, with frequencies >0.03, were identified. The results were obtained in the absence of any assumptions regarding the biological feasibility of any particular genotype, and without specification of the multiplicity of each individual infection. The model enables consistent analysis of actual frequencies, and allows use of all of the data collected. Moreover, it offers improved speed and versatility compared with some existing statistical methods of malaria genotype frequency estimation. These results may have key implications for the surveillance of antimalarial drug resistance, including the use of prospective markers of artemisinin resistance.

## 1.5.5 Pathogenesis of severe malaria

### O.1.5.5.002
**Analyzing *Plasmodium falciparum* erythrocyte membrane protein 1 gene expression by a next-generation-sequencing based method**

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*Plasmodium falciparum* is the parasite responsible for most cases of severe malaria and causes >1 million deaths every year. The particular virulence of this Plasmodium subspecies is highly associated with the expression of certain members of the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family, encoded by approximately 60 highly variable var genes in each parasite genome. PfEMP1 is exported to the surface of infected erythrocytes and is thought to be fundamental to immune evasion by adhesion to host- and parasite factors. The highly variable nature has constituted a roadblock in var expression studies aimed at identifying PfEMP1 features associated with severe pathogenesis. Here we present the first effective method for characterization of var genes expressed in the field: a sequential PCR and next-generation-sequencing based technique applied on (i) an expressed var sequence tag (approximately 400 bp) present in most vars and subsequently on (ii) near-full length var genes (5–12 kb), which are targeted based on the expression levels detected by (1). The method has been validated and the results obtained from field samples lend support to quantitative PCR studies showing vars of the group A and domain cassettes 8 and 13 types to be expressed at high levels in severe childhood malaria.

### O.1.5.5.003
**Evaluating the role of a novel human receptor in severe malaria**

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There has been an ongoing search for host receptors responsible for the development of severe malaria infections. We have recently discovered a new host receptor to which *Plasmodium falciparum* infected Erythrocytes expressing a subset of *Plasmodium falciparum* Erythrocyte Membrane 1 (PfEMP1) proteins, associated with severe disease outcome, bind. We have found that adhesion to primary brain microvascular endothelial cells of parasites isolated from Tanzania was higher in patients with severe complications compared to parasites isolated from patients with mild and uncomplicated disease. We aim to further establish the link between binding of infected erythrocytes to the new receptor and the clinical outcome of *Plasmodium falciparum* malaria infection by deploying adhesion assays in malaria endemic countries. Parasite binding assays used for initial studies on *in vitro* cultured lines are presented along with latest development of parasite binding assays for studies of the adhesion phenotype of freshly isolated infected erythrocytes. These binding assays are important tools for studies of malaria pathogenesis as well as for vaccine testing.

## 1.5.6 Malaria in pregnancy

### O.1.5.6.001
**High level *Plasmodium falciparum* sextuple dihydrofolate reductase/dihydropteroate synthetase is associated with reduced birth weight**

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BACKGROUND Pregnancy associated malaria (PAM) caused by *Plasmodium falciparum* is known to affect pregnancy outcomes

and it has been implicated to low birth weight, miscarriage, intrauterine growth retardations and maternal anaemia, among others. Current PAM control strategy such as intermittent preventive treatment in pregnancy using sulfadoxine pyrimethamine (IPTp-SP) is compromised by widespread drug resistance due to single nucleotide polymorphisms in the *P. falciparum* dihydrofolate reductase (Pfdhfr) and the dihydropteroate synthetase (Pfdhps) genes.
METHODS A cohort of 924 pregnancy women was longitudinally followed up from inclusion until delivery in Korogwe District, northeastern, Tanzania. *P. falciparum* parasites were genotyped for molecular markers related to SP resistance in the dhfr and dhps genes. Correlation between these molecular markers with newborn's birthweight was assessed by linear regression models.
RESULTS We showed that the parasite population in this particular setting is highly saturated with dhfr and dhps parasites genotypes. Interestingly, the highly mutated parasites with three level mutations in both dhfr and dhps genes (sextuples) had a significant reduction in birthweight as compared to the least mutated haplotypes, 359 g (95% CI: −601, −117); $P = 0.005$.
CONCLUSION AND INTERPRETATIONS The fact that the highly mutated parasite genotypes had a significant effect on newborn's birthweight underscore the needs for early identification of these cases through diagnosis with accurate and reliable diagnostic tools and treatment with safe and effective antimalarial drugs. IPTp-SP might be sub-optimal in this particular setting with high resistance and declining transmission.

### O.1.5.6.002

**Efficacy and safety of mefloquine as malaria intermittent preventive treatment in pregnancy (IPTp): results from a multicenter randomized trial**

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The current recommendation by the World Health Organization (WHO) to prevent malaria infection in pregnancy in areas of stable malaria transmission relies on the prompt and effective case management of malaria illness, the use of intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) at each scheduled antenatal clinic (ANC) visit and the use of insecticide treated nets (ITNs). The medium and long-term use of SP for IPTp has raised concerns given the increase in SP resistance in parasites. The evaluation of alternative antimalarials for IPTp is thus urgently needed. Of all the available alternative antimalarial drugs mefloquine (MQ) is the one that offers the most comparative advantages to SP. A randomized, open-label, superiority 3 -arm trial was conducted in four African countries (Benin, Gabon, Tanzania and Mozambique) to compare 2-dose MQ versus 2-dose SP for IPTp in the prevention of the adverse effects of malaria during pregnancy and to compare MQ tolerability of two different MQ administration regimens. The three arms of the study were: (i) IPTp with SP, (ii) IPTp with MQ given as full dose, (iii) IPTp with MQ split dose given over 2 days. All the trial participants received a long-lasting ITN at recruitment and were followed-up until the children reached age one. In total 4750 pregnant women were enrolled at the ANC from September 2009 to January 2012. The study results on the efficacy and safety of MQ and SP as IPTp shall be presented.

### O.1.5.6.003

**Sulfadoxine pyrimethamine for malaria prophylaxis among antenatal care mothers in Zambia: an observational study**

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BACKGROUND Sulfadoxine Pyrimethamine (SP) still remains the drug used for intermittent preventive treatment (IPTp) for malaria in Zambia. However, although there is documented increase in resistance to SP, it still remains the mainstay of IPTp medication at all antenatal care services in Zambia.
MATERIALS AND METHODS An observational cohort study in Zambia assigned pregnant women attending antenatal clinics to groups based on the drug used to treat their most recent malaria episode [Coartem® (AL) versus sulphadoxine-pyrimethamine (SP)] for prospective follow-up of pregnancy related outcomes. Based on this, the SP IPTp intake status of these patients were analysed to explore whether adherence to SP IPTp had any impact on the outcome of malaria in the current pregnancy. All the enrolled women were followed up until 6 weeks post delivery and 12 months for the live births.
RESULTS The observational cohort had 1001 pregnant women and 66 of those who received IPTp after enrolment reported malaria attacks. Of these 28 out of 66 (42%) received either one or two doses of SP IPTp but had an episode of malaria. Additionally, 39 out of 66 (59%) had two doses of SP IPTp but out of these 17 (44%) had an episode of malaria. (More results will be available at the time of the congress).
CONCLUSION There is need to explore the efficacy of SP IPTp among pregnant women in the light the ever increasing threat of SP resistance. To review the issue of compliance to SP IPTp and document breakthrough malaria episodes over time in view of the changing epidemiology of malaria in Zambia.

## 1.5.7 ACTs and new anti-malarials

### O.1.5.7.001

**Anti-malarial activity of *Orostachys japonicus* (Crassulaceae) against 3D7, K1 strains, and field isolates (Madang, Papua New Guinea) of *Plasmodium falciparum*: identification of 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one as a potent inhibitor of PfM18AAP**

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INTRODUCTION In times of malaria prevalence on the Korean peninsula, *Orostachys japonicus* (*O. japonicus*) (Crassulaceae) extract was often used to cure the infected patients. The present study thus aimed to examine the anti-plasmodial activity of the plant extract and to isolate major active compounds.
MATERIAL AND METHODS *O. japonicus* ethanol extract and a total of five organic solvent-soluble portions separated from the extract were tested *in vitro* against 3D7 and K1 strains of *Plasmodium falciparum* (*P. falciparum*) using HRP2-based ELISA. Among the five portions, dichloromethane-soluble portion was subjected to GC-MS analysis. The identified major compounds were isolated by preparative HPLC and were tested for their anti-plasmodial activities. The most active compound of all was then examined for its possible hemolytic and cytotoxic activities. The compound's inhibitory effects on plasmodial growth were assessed *in vivo* against *Plasmodium berghei*, and on field isolates from Madang, Papua New Guinea as well.


 

Inhibition of PfM18AAP activity by the identified compound was assessed using enzymatic inhibition assays.
RESULTS *O. japonicus* ethanol extract exhibited a promising activity. Among its organic solvent-soluble portions, dichloromethane-soluble portion was found to be the most active one. From the dichloromethane-soluble portion, three major compounds were isolated, one of which showed an excellent anti-plasmodial activity (IC50 < 1 $\mu$g/ml). The compound was identified as 3-(benzylamino)-4-ethyl-6-phenyl-1,2,4-triazin-5-one. The compound had very low hemolytic and cytotoxic activities, and was highly effective against field isolates. The compound yielded significant inhibition rates *in vivo*. Moreover, the compound was identified as a potent inhibitor of PfM18AAP, and a hypothesis concerning the compound's mechanism of action was drawn from the observed result.
CONCLUSIONS The highly promising anti-malarial activity exhibited by its major active compound led to the conclusion that *O. japonicus* holds a great deal of promise as a candidate for the source of a new anti-malarial drug.

## O.1.5.7.002

### Treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether–lumefantrine: Impact on the prevalence of anemia

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INTRODUCTION Malarial anemia is an important public health problem in malaria-endemic countries of Africa. Effect of systematic treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether-lumefantrine (AL) on hemoglobin (Hb) levels and anemic status, compared with no treatment of asymptomatic carriers, was investigated.
METHODS AND MATERIALS In a 12-month, single-center, controlled, parallel, cluster-randomized study in 18 villages in Burkina Faso, inhabitants of villages randomized into intervention and control arms participated in three community-screening-campaigns (CSC1–3) that took place approximately 1 month apart before the rainy season, and a fourth campaign (CSC4) after the rainy season at study end (i.e. at 12 months).
RESULTS Change in Hb level in all asymptomatic carriers aged >6 months from Day 1 to Day 28 of CSC1 was +0.53 g/dl (from 11.81 to 12.33 g/dl) in intervention arm versus −0.21 g/dl (from 12.06 to 11.86 g/dl) in control arm ($P < 0.001$). During the same period, the proportion of asymptomatic carriers aged >6 months to <5 years with anemia (mild, moderate or severe) decreased by 31.1% (from 75.7% to 44.6%) in intervention arm, compared with a decrease of 4.7% (from 76.3% to 71.6%) in control arm. The proportion of asymptomatic carriers with anemia was reduced in both arms after 12 months.
CONCLUSION Systematic screening and treatment of asymptomatic carriers of *P. falciparum* with AL at the community level can reduce the prevalence of anemia in children in the short term, although difference with the control arm was not maintained at 12 months.

## O.1.5.7.003

### Open source drug discovery for malaria

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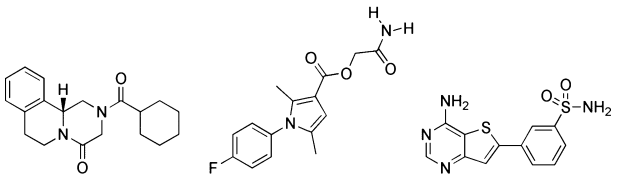
INTRODUCTION Open source drug discovery operates on the principles that all data and ideas in a project should be freely shared, anyone may participate in the project at any level and there will be no patents. By working in this manner, research is accelerated through the recruitment of expertise from a variety of participants.
MATERIALS AND METHODS Synthetic chemistry and biological evaluations are recorded using online electronic laboratory notebooks. Project coordination is effected through the use of online project management tools, and participants are encouraged to join the project through social media.
RESULTS This presentation will describe two open projects that have been conducted in this way. The first, a collaboration with the World Health Organisation, discovered an inexpensive route to the active enantiomer of praziquantel, the drug of choice for the treatment of schistosomiasis and is described to illustrate the approach. The second project, a collaboration with the Medicines for Malaria Venture, involves a hit-to-lead campaign in antimalarial drug discovery, using the GlaxoSmithKline Tres Cantos open dataset as a starting point. Scientific progress on two series will be described. The first, based on the arylpyrrole TCMDC 123812, discovered some potent novel compounds that performed well in a late stage gametocyte assay, but which ultimately suffered from either hydrolytic lability or poor solubility. A second series, based on the aminothienopyrimidine TCMDC 135294, is currently under development and while the initial hit has shown excellent potency and drug-likeness, potency is easily eliminated through simple structural variations.
CONCLUSIONS Open projects can discover potent novel molecules through a massively distributed collaboration. They benefit from inputs derived from people outside the core teams. Illustrations of this will followed by some concluding remarks on the feasibility of drug discovery without patents.

Structures



## O.1.5.7.004

### Discovery of a new generation of fast-acting antimalarials

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Malaria is the world's most deadly parasitic disease and one of the most severe public health problems worldwide. Currently, artemisinin-based combination therapies (ACTs) are the preferred treatments for *P. falciparum* malaria because of the fast acting mode of action of endoperoxides. ACTs treatments are compromised by the advance of resistance therefore identification of new fast-acting molecules is a crucial objective when looking for artemisinin replacements. Fast-acting agents should maximize the therapeutic efficacy and minimize the potential to induce resistance to new treatments. Standard assays used to assess antimalarial potency of compounds are based on parasite metabolism measurement. However, these methods are not adequate to evaluate true effects over parasite viability and hence, to measure the speed-of-action of compounds. In that regard, a new *in vitro* methodology, Parasite Reduction Rate assay (PRR) (PLos One, 2012) allows assessing viability of drug-treated parasites over time and would enable effective identification of fast-acting antimalarials. At GSK, we have profiled the entire corporate screening collection (>2 million compounds) in a *P. falciparum* whole cell assay. This initiative created the Tres Cantos Antimalarial Set (TCAMS) which comprises more of 13K novel hit compounds that are freely available to the global drug discovery community. In this poster we describe how the PRR assay has been efficiently used to identify compounds with a fast-acting mode of action within TCAMS. More than one hundred hits have been progressed through this assay. Results have allowed us to identify molecules with a fast-acting antimalarial mode-of-action that could potentially enable the discovery of novel antimalarials to replace artemisinin in current front-line treatments. Some of these molecules are currently the object of lead optimization programs that hopefully will deliver a new generation of antimalarial drugs.

## O.1.5.7.005

### Impact of ACT scale-up on fever case management in the public and private sectors in Africa: evidence from nationwide surveys

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BRIEF INTRODUCTION In recent years initiatives such as the pilot phase of the Affordable Medicines Facility – malaria (AMFm) have proved to be a 'game changer' for the private sector, resulting in increased availability and reduced price of quality-assured ACT in for-profit outlets. Given the well-documented role of the private sector as a treatment source in sub-Saharan Africa, we investigate whether increased availability of affordable ACT is associated with improvements in case management of suspected malaria.
MATERIAL AND METHODS As part of the ACTwatch program nationally-representative household surveys focused on treatment-seeking behaviour for fever among children under five were conducted in Benin, Madagascar, Nigeria, Uganda and Zambia between 2009 and 2010, and repeated in 2011/2012. Detailed information on treatment-seeking behaviour was collected, including where advice and treatment was sought, and diagnostic services and medicines received at each source. Treatment indicators were tabulated by sector across countries; differences in case management provided by each sector over time were examined using logistic regression.
RESULTS Increases in presumptive ACT use among children with fever were seen in all countries, ranging from 5% points in Madagascar (3–8%) to 24% points in Uganda (20–44%). When restricted to children who received any antimalarial the increases in ACT use were even greater, from 7% to 45% in Madagascar and 40–83% in Uganda, for example. These results will be further disaggregated by source of treatment to facilitate comparison with outlet survey data. During the same period in Uganda, overall availability of ACT increased from 34% to 75%, with the majority of this increase coming in the private sector.
CONCLUSIONS Results on appropriate case-management for fever will be contextualised in light of findings from contemporaneous outlet surveys also conducted by ACTwatch. Differences between the public and private sectors will be discussed.

## O.1.5.7.006

### Dihydroartemisinin-piperaquine versus artemether-lumefantrine for first-line treatment of uncomplicated malaria in African children: a cost-effectiveness analysis

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Recent trials in Africa showed that dihydroartemisinin-piperaquine (DHAPQ), a newer fixed-dose artemisinin-based combination therapy (ACT), was as efficacious and safe as artemether-lumefantrine (AL) for treatment of young children with uncomplicated malaria across different endemicity settings. Longitudinal follow-up of patients also confirmed that DHAPQ had a longer post-treatment prophylactic effect than AL, reducing the risk of recrudescence and reinfection and hence conferring additional health benefits to patients. We estimated the threshold costs of DHAPQ per course treatment for which DHAPQ would be a cost-effective alternative to AL, given the post-treatment prophylactic effects of these drugs. Decision analysis was performed using a Markov model that simulated uncomplicated and severe malaria incidence, survival, disability adjusted life years (DALYs) and costs in a hypothetical cohort of children receiving DHAPQ or AL for treatment of uncomplicated malaria. We calculated weekly hazard rates of recurrent malaria post-treatment for the two treatment groups, using the published data on the proportion of patients whose treatment was failure free by day of follow up from a multicenter randomized control trial in Africa. At a mean cost of $0.36 per treatment course for both drugs, first-line treatment with DHAPQ was the dominant treatment strategy over AL with an improvement of 0.05 DALYs averted per child (95% CI 0.01–0.12) and a cost saving of $2.06 per child (95% CI 0.48–5.09). First-line treatment with DHAPQ remained the dominant treatment strategy (less costly and more effective) for any cost per course treatment below $1.27. Between $1.27 and $1.78, first-line treatment with DHAPQ was a 'highly attractive' intervention compared to AL with an incremental cost-effectiveness ratio '<$25 per DALY averted' under World Bank guidance. This study demonstrates the superiority of DHAPQ over AL for the treatment of uncomplicated *P. falciparum* malaria in young children from a clinical and economic perspective.

## 1.5.8 Malaria epidemiology: burden and causes

### O.1.5.8.001

**Declining burden of malaria over two decades in a rural community of Muheza district, north-eastern Tanzania**

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BACKGROUND The declining burden of malaria in some Africa countries has been attributed to scaling-up of different interventions. This study assessed the long term trends of malaria burden for 20 years in Magoda and 15 years in Mpapayu village of Muheza district, Tanzania, in relation to different interventions and changing malaria control policies.
METHODS Cross-sectional surveys recruited individuals aged 0–19 years and blood smears were collected for detection of malaria parasites by microscopy. Prevalence and density of *Plasmodium falciparum* infections and other indices of malaria burden (prevalence of splenomegaly and gametocytaemia) were compared across the years and between the study villages.
RESULTS In Magoda, the prevalence of *P. falciparum* infections initially decreased between 1992 and 1996 (from 83.5% to 62.0%), stabilized between 1996 and 1997, and further declined to 34.4% in 2004. A temporary increase between 2004 and 2008 was followed by a progressive decline to 7% in 2012. In Mpapayu, the highest prevalence was 81.5% in 1999 and it decreased to 25% in 2004. After a slight increase in 2008, a steady decline followed, reaching <5% from 2011. Bed-net usage was high in both villages from 1999 to 2004 (≥88%) but it decreased between 2008 and 2012 (range, 28–68%). After adjusting for the effects of bed-nets, age, fever and year of study, the risk of *P. falciparum* infections decreased significantly, by ≥97% in both villages between 1999 and 2012 ($P < 0.001$). The prevalence of splenomegaly (>40–<1%) and gametocytaemia (23–<1%) also decreased markedly in both villages.
DISCUSSION AND CONCLUSIONS A remarkable decline in the burden of malaria occurred between 1992 and 2012. The initial decline (1992–2004) was possibly due to the deployed interventions while the steady decline observed from 2008 (with bed-net coverage) suggests that other factors contributed to these changes.

### O.1.5.8.002

**Do malaria vector control measures impact disease-related behaviors and knowledge? Evidence from a large-scale larviciding intervention in Tanzania**

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INTRODUCTION Recent efforts of accelerated malaria control towards the long-term goal of elimination had significant impacts in reducing malaria transmission. Yet, high coverage of interventions in low transmission settings needs to be maintained. Reducing transmission, however, could negatively impact disease risk perception, hinder adherence to protective measures, and affect disease-related knowledge. The goal of this study was to investigate the potential impacts of interventions aimed at reducing vector density on bednet usage and malaria-related knowledge.
MATERIAL AND METHODS Dar es Salaam's Urban Malaria Control Program was launched in 2004 with the aim of developing a sustainable community-based larviciding intervention. After 2 years of baseline data collection, larviciding was implemented in three out of 15 study wards and subsequently scaled-up, in two other phases, to the whole study region. Socio-demographic data was collected using a randomized cluster sampling design (2004–2008). Prevalence ratios (PR) for the effect of the larviciding intervention on bednet usage ($N = 64\,537$) and household heads' knowledge of malaria symptoms and transmission ($N = 11\,254$) were obtained from Bayesian logistic regression models.
RESULTS The probability that individuals targeted by larviciding had used a bednet wad 5% time less than that of the control areas [PR = 0.95; 95% Credible Intervals (CrI): 0.94–0.97] and the magnitude of this effect increased with time. Larviciding also led to a decline in household heads' knowledge of malaria symptoms (PR = 0.88; 95% CrI: 0.83–0.92) and this effect was more pronounced for individuals of low SES. We found that the intervention had no effect on knowledge of malaria transmission (PR = 1.00; 95% CrI: 0.95–1.05).
CONCLUSION With renewed impetus towards the long-term goal of elimination, our study suggests that reducing vector density and malaria transmission could pose additional challenges. We conclude that community engagement, locally-adapted educational strategies, and media campaigns should be considered in order to mitigate such negative impacts.

### O.1.5.8.003

**The role of environmental data in forecasting malaria in Uganda**

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INTRODUCTION Accurate predictions of malaria could provide public health and clinical health services with an opportunity to develop proactive, targeted approaches for malaria control and prevention that make effective use of limited resources. The objective of the research was to develop and evaluate the accuracy of malaria forecasting models for six different sentinel sites in Uganda, using environmental predictors.
MATERIAL AND METHODS Health clinic data collected by the sentinel site surveillance program, Uganda Malaria Surveillance Project, was used in conjunction with satellite-derived rainfall, temperature, and vegetation estimates. Weekly, site-specific time series were created and examined for all variables. The potential lag between each predictor and confirmed malaria was incorporated into the Autoregressive Integrated Moving Average models. We generated a set of short-term, intermediate, and long-term forecasts of malaria prevalence at weekly intervals and the average forecast error was calculated, using a reserved portion of the training series.
RESULTS The temporal interaction of the environmental predictors and malaria ranged from 1 week to 4 months. The significance of rainfall, temperature, and vegetation as predictors differed across the sites, although generally, temperature and vegetation were better predictors of malaria when compared to

rainfall. The forecasting accuracy improved with the series stratified by age group and the environmental predictors were significant for malaria prevalence for ages 0–5 and 5–15 but less often significant for ages 15 and over. The forecasting accuracy deteriorated with increased forecasting intervals and the short-term forecast accuracy ranged from 5% to 29% across the sites (mean absolute percent error).
Conclusions Our work demonstrated the utility of using environmental covariates in the prediction of malaria, when used in conjunction with historical counts of malaria. Future work includes examining the predictive influence of treatment and malaria screening practices which we anticipate will improve the forecast accuracy of our models.

### O.1.5.8.004

**Evaluating changing malaria transmission in a rural village, in coastal Tanzania, by assessment of IgG antibodies against *Plasmodium falciparum* and *Anopheles gambiae* antigens**

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Introduction The changing epidemiology of malaria in Sub-Saharan Africa over the last decade needs further understanding. A longitudinal project on the epidemiology of malaria was set up in the Nyamisati village, Rufiji District, Tanzania in 1985. Cross-sectional surveys have revealed a gradual decrease in parasite prevalence over the last 25 years, from >90% to 13% (by PCR), despite the absence of large scale control interventions. Although PCR provides good detection of low-level parasitemia, assessment of parasite prevalence alone may not fully reflect the level of exposure in a population. In order to further understand the long-term dynamics of malaria transmission in this setting, we combined an assessment of parasite prevalence with analyses of IgG antibodies against antigens of the *P. falciparum* parasite and a salivary antigen of the Anopheles vector.
Material and Methods The study included children 1–16 years old participating in cross-sectional surveys in Nyamisati village in 1995, 1999 and 2010. Parasite prevalence was established by PCR. Detection and quantification of IgG antibodies against *P. falciparum* crude schizont extract (PfSE) and Anopheles gambiae salivary gland protein 6 (gSG6) were performed with enzyme-linked immunosorbent assay (ELISA). Levels of IgG antibodies against merozoite surface protein 1–19 (MSP-119) and merozoite surface protein 2 (MSP-2) were assessed using a multiplexed bead-based immunoassay.
Results and Conclusions Parasite prevalence and the prevalence and levels of IgG antibodies against PfSE, MSP-119 and MSP-2 decreased between 1999 and 2010, with the most marked reduction among the youngest children (1–4 years of age). The prevalence and levels of anti-gSG6 IgG antibodies showed a similar pattern. These findings suggest a substantially decreased intensity of malaria transmission in the Nyamisati area over the past 10 years, together with an evident decrease in the population level of exposure to the malaria vector.

### O.1.5.8.005

**Spatio-temporal clustering of clonal parasites in recurrent vivax malaria infections after radical cure treatment in the Peruvian Amazon**

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Introduction Despite the large burden of *Plasmodium vivax*, little is known about its transmission dynamics. The study of the parasite population structure and dynamics give insights on particular epidemiological features of malaria in endemic areas. We explored the population structure and spatio-temporal dynamics of *P. vivax* infections in a 2-year cohort study carried out in a rural community of the Peruvian Amazon.
Materials and Methods Between March and December 2008, 37 *P. vivax* patients were treated with chloroquine and primaquine and followed up monthly for 2 years with systematic blood sampling. All samples were screened by microscopy and species-specific PCR for malaria parasites and subsequently all *P. vivax* infections genotyped using 15 microsatellites. Parasite population structure and dynamics were determined by computing different genetic indices and using spatio-temporal statistics.
Results Seventy-six percent of the study participants experienced one or more recurrent *P. vivax* infections during their follow-up. Fifty-five percent of the recurrences were sub-patent and asymptomatic. The *P. vivax* population displayed limited overall genetic diversity (He = 0.49), high frequency of monoclonal infections (84%), presence of linkage disequilibrium (LD) and low probability of outbreeding (Psex<0.0001). Up to 20 unique haplotypes unequally distributed in four haplogroups were found. Haplotype replacement was reflected by spatio-temporal clustering and changes on the degree of LD and the genetic diversity after the first year of follow up ($P < 0.001$).
Conclusions The parasite population and dynamics found in this study may explain some epidemiologic features of the study area: (i) the clonal parasite population and haplotype clustering could be favored by the geographical isolation of the study area; (ii) continuous infections with the same clones may facilitate the development of clinical immunity, reflected on high recurrence of asymptomatic infections; (iii) the high rate of recurrent infections after treatment could be associated to the increased risk of spread of drug resistance traits in clonal populations.

## 1.5.9 Malaria diagnosis

### O.1.5.9.001

**Development and evaluation of a sensitive direct-on-blood PCR – lateral flow assay for the near point of care diagnosis of malaria**

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Molecular tools allow for specific/sensitive malaria diagnosis, but current formats, like PCR with gel-electrophoresis, are difficult


 

to implement in resource poor settings. Therefore, a simple, fast, sensitive/specific molecular diagnostic platform, direct on blood PCR combined with nucleic acid lateral flow immunoassay (NALFIA) to detect amplified PCR products of Plasmodium species and human GAPDH (internal control) was developed and evaluated under laboratory conditions, a multi country ring trial and in two malaria endemic countries (Burkina Faso and Thailand). Analytical sensitivity/specificity of PCR-NALFIA in a single laboratory evaluation was >95% and the test was able to detect <1 parasite/$\mu$l blood. All four laboratories in the ring trial reported ease of use of the system and could successfully perform the protocol. Overall laboratory inter variability was low and the agreement of reported results was high. Overall k-value was 0.89 (95% CI: 0.83–0.94; $P < 0.001$). Overall test sensitivity and specificity was >95% with very small confidence intervals. Field evaluations by local staff without prior training in performing the dbPCR-NALFIA in disease endemic countries, Thailand and Burkina Faso, were performed. In Burkina Faso (*P. falciparum* environment) the relative sensitivity was 94.8% and relative specificity 82.4% compared to microscopy and 93.3% and 91.4% compared to RDT. In Thailand (*P. vivax* environment) the relative sensitivity and relative specificity was 93.4% and 90.9% respectively compared to microscopy and 95.6% and 87.1% compared to RDT. These numbers are under estimation of test performance as the results are not PCR corrected.
FUNDING EU FP7 grant 201889 Multi drug resistance in malaria under combination therapy: assessment of specific markers and development of innovative rapid and simple diagnostics (MALACTRES).

## O.1.5.9.002
### Evaluation of gametocyte detection in different patient material

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INTRODUCTION For future eradication strategies of malaria, it is important to control the transmission of gametocytes from man to the anopheline vector that causes spreading of the disease. A sensitive, ideally non-invasive, field-proven method is needed.
MATERIAL AND METHODS Microscopically *Plasmodium falciparum* positive patients from Jimma, Ethiopia, donated finger prick, venous blood, saliva, oral mucosa and urine samples that were spotted on filter paper or swap. All samples were taken and stored under the same conditions. RNA was extracted from filter paper and detected by real-time QT-NASBA. Pfs16-mRNA and Pfs25-mRNA were measured in time to positivity (TTP) to detect gametocyte specific mRNA in different gametocyte stages and were compared to 18S-RNA that is expressed in all parasite stages. Results were quantified via dilution series of artificial RNA and parasite cultures.
RESULTS Ninety-six samples of 16 uncomplicated malaria patients were investigated. For all targets, molecular detection in finger prick samples was most sensitive, even when compared to venous blood samples. Results for pfs16 and pfs25 showed evidence for a real difference ($P = 0.03$ and $P = 0.02$, respectively). Detection of 18S-RNA in saliva and urine showed sensitivities of 80% and 67%, respectively.
CONCLUSIONS Sensitivity of non-invasively collected material like urine, saliva or mucosa seems unsuitable for the detection of gametocyte-specific mRNA. Gametocyte detection sensitivity in asymptomatic carriers might be generally even lower. As suspected, finger prick testing was the most sensitive method and should preferably be applied in future transmission control and eradication plans. A rapid test for gametocyte targets would simplify efforts.

## O.1.5.9.003
### Early diagnosis of malaria using malaria rapid test Pf/Pan (HRP2, pLDH) in Thailand

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Malaria caused by the Plasmodium species has resulted in significant health problems in Thailand. The *Plasmodium falciparum* is especially a major cause of global morbidity and, in rare cases, mortality, particularly to those people in remote areas. Rapid detection and prompt treatment is a promising model accepted among malarious countries by using Malaria Rapid Diagnostic Tests (MRDT). We launched Standard Diagnostics (SD) Point of Care Test (POCT) for Malaria detecting either *P. falciparum* or non-*P. falciparum* in the malaria control of Thailand. Two hundred and ninety-nine suspected cases of malaria, who attended three Malaria Clinics in Kanchanaburi, Ranong and Tak Provinces, were recruited. of those cases, 37.8% (113) were children under 15 years old. The results showed that the MRDT revealed sensitivity to *P. falciparum*, non-*P. falciparum* and specificity at levels of 95.29, 97.57 and 98.21 respectively. Moreover, it represented in similar trend when the children were separated, this test revealed 95.88, 95.00 and 100 sensitivity to *P. falciparum* and non-*P. falciparum* specificity respectively. This MRDT showed high diagnostic values and could be used in any malaria endemic areas. In addition, it provides great benefit in detecting malaria in children.

## O.1.5.9.004
### Adherence to rapid diagnostic test results among community medicine distributors in rural Ugandan communities

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BACKGROUND The WHO recommends universal access to malaria diagnostics, and malaria rapid diagnostic test (mRDT) is the only feasible test at community level. Evidence regarding adherence to mRDT results by community medicine distributors (CMDs) and feasibility for use in community case management (CCM) remains limited. We assessed adherence to mRDT results by CMDs in rural Uganda, to provide information that could guide mRDT-based CCM to avoid overuse of artemisinin-based combination therapy (ACTs).
METHODS A cluster-randomised trial was undertaken to examine the feasibility and cost-effectiveness of mRDT use by CMDs in two areas with differing malaria transmission. In each setting, communities were randomised to one of two arms: ACT treatment following mRDT testing (intervention arm) was compared with presumptive treatment (control arm). Data on diagnosis and treatment were recorded by CMDs in treatment registers. Household follow-up interviews and focus group

discussions were conducted with CMDs and caretakers of under-5 children.
RESULTS Overall, adherence to mRDT results by CMDs exceeded 85% in both transmission settings. In the high transmission area, adherence to treatment guidelines was high as only 1% of children with mRDT-negative results were treated with an ACT. As a consequence, 44% of children seen by CMDs in the mRDT arm compared with 99% of patients in the presumptive arm were treated with an ACT, reducing ACT treatment by 55%. Similarly, in the low transmission area, only 3% of children with mRDT-negative results were treated with an ACT. Under conditions of low transmission, <10% of children in the mRDT arm overall were treated with an ACT compared with 94% in the presumptive arm, reducing ACT prescription by 87%.
CONCLUSION Training CMDs in use of mRDT in the context of CMM is feasible. CMDs performed well and adhered to malaria treatment guidelines thus improving rational use of ACTs.

## O.1.5.9.005

### A cluster randomised trial introducing rapid diagnostic tests into the private health sector in Uganda: mpact on appropriate treatment of malaria

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INTRODUCTION The WHO recommends universal access to malaria diagnostics, encompassing all treatment providers, including the private sector. Rapid diagnostic tests (RDTs) may provide a simple means of confirming malaria diagnosis in drug shops. As yet, there is little evidence of the impact of diagnostic testing on antimalarial drug sales and referral practices by drug shops, particularly in Africa.
METHODS A cluster-randomised trial to evaluate the impact and cost-effectiveness of using RDTs in registered drug shops, compared with presumptive treatment, has been conducted in Mukono District, Uganda since October 2010. The trial aimed to evaluate the impact of diagnostic testing on the proportion of drug shop clients who receive appropriate ACT treatment, in line with parasitological status as defined by malaria microscopy. A total of 65 drug shops were randomised to receive training either in the use of RDTs or presumptive diagnosis of malaria. All drug shop vendors (DSVs) were trained on the national malaria treatment guidelines, use of rectal artesunate pre-referral treatment, and when to refer. Supporting interventions included activities to raise community awareness to emphasise that not all fevers were malaria and that there was need to test blood before receiving or purchasing an ACT. DSVs received close support supervision for 2 months of implementation.
RESULTS Introduction of RDTs in drug shops was acceptable to DSVs, the community and health staff. Adherence to RDT results by DSVs was high with 92% of treatment decisions being consistent with RDT test results. The intervention reduced sales of ACTs by approximately 40%, compared to drug shops in the control arm (presumptive diagnosis). Drug shops using RDTs were more likely to refer patients and referral was mainly reported for RDT-negative clients.
CONCLUSION Introducing RDTs in drug shops was feasible and acceptable; and had a substantial impact on adherence and referral of RDT-negative patients.

## O.1.5.9.006

### Restricting artemisinin-based combination therapy to test positive malaria in a high-transmission setting in Ghana – a cluster-randomised trial

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INTRODUCTION Following WHO recommendations, test and treat malaria policy is replacing presumptive treatment in many endemic countries. However there is very limited data on the effect of test and treat policy on the incidence of malaria, anaemia and severe febrile illness in high-transmission settings.
METHODS We conducted a cluster randomized trial to compare the effect of test and treat versus presumptive treatment in a high transmission setting in Ghana. Thirty two health centres were randomly allocated to test treat (TT) or presumptive treatment (PT) arms. Children aged 2–24 months living around health centres were enrolled and the incidence of malaria, anaemia and severe febrile illness were measured by passive and active surveillance over 24 months.
RESULTS A total of 3046 children were enrolled in 32 health centres. The incidence of malaria in the TT arm was 696/1000 pyrs (95% CI 557, 870) and 800/1000 pyrs (700, 914) in the PT arm ($P = 0.56$). The incidence of first or only episode of malaria after the first episode of febrile illness was 558/1000 pyrs (95% CI 434, 718) in the TT arm and 673/1000 pyrs (544, 833) in the PT arm ($P = 0.34$); the incidence of anaemia was 82/1000 (63, 107) vs. 92/1000 (71, 119) ($P = 0.61$); incidence of severe febrile illness was 129/1000 (82, 210) vs. 151/1000 (95, 250) ($P = 0.84$). Children in the PT arm were more likely to be prescribed ACT than children in the TT arm (81.6% vs. 72.1%; $P = 0.01$).
CONCLUSION Restricting the use of artemisinin combination therapy to test positive malaria is appropriate even in medium-high transmission settings.

## O.1.5.9.007

### The community microscopist impact on malaria control in remote populations of the Brazilian Amazon

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INTODUCTION Early parasitological diagnosis and treatment have unquestionable impact in malaria control. In many remote areas, however, disease control is hampered by the lack of access to detection and treatment. The authors aimed to evaluate the impact of training community microscopists in an area of the Brazilian Amazon where its remoteness condition discourages the implementation of usual control measures.
METHODOLOGY The Jau's National Park (JNP) is a large remote area of malaria transmission in the Amazonas State, Brazil (2 272 000 ha and 0.04 inhabitants/km$^2$). Between 2001 and 2005 information, education and communication activities were implemented along with the local population. In 2008 a resident from each of the eight communities was trained to diagnose malaria and give specific treatment, according to the Ministry of


 

Health protocol. Since then, they have been periodically evaluated, retrained and replaced (when necessary) to promote self-confidence and reliability. In 2008, the community microscopist figure was introduced and one centre per community was equipped with microscope, consumables, solar panels and one electric boat. The time between the first symptom and the diagnosis and treatment together with the number of malaria cases per community was evaluated before and after the intervention.
RESULTS In 2007 were reported 455 malaria cases (279 *Plasmodium vivax*, 182 *P. falciparum* and 14 mixed infections) and in 2011 were reported 12 malaria cases (nine *P. vivax* and three *P. falciparum*). In 2007, in the great majority of cases (91.39%) the time between the first symptom and diagnosis and treatment took 48 h or more. In 2010, in only 11.9% of cases it took 48 h or more and in 61.66% <24 h.
DISCUSSION AND CONCLUSIONS This work shows that community microscopists can play an important role in the control of malaria in areas without access to usual malaria control actions.

## 1.6 Antibiotic Resistance in Man and Animals

## Antibiotic resistance, quality of antibiotic drugs and antibiotic misuse: A curse for health in Africa?

### O.1.6.002

**Antibiotic prescription practices in selected health care facilities in Eastern Region, Ghana**

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INTRODUCTION Increasing microbial resistance remains a global challenge and a threat to public health, especially in low-income countries. Irrational prescription and use of antibiotics is reported as the most important cause of microbial resistance. In Ghana, over 40% of patients attending public healthcare facilities receive an antibiotic while 80% of non-pneumonia acute respiratory infections are treated with antibiotics. A careful study of factors motivating irrational prescribing of antibiotics is therefore required in developing promising interventions.
METHODS In-depth interviews were conducted with 13 prescribers in four selected healthcare facilities in the Eastern region of Ghana in April 2011. Twelve patient-prescriber consultations were also observed using an observational guide. In-depth interviews were transcribed verbatim, observational notes incorporated and transcripts analysed by template analysis.
RESULTS Prescribers mentioned several factors that they perceived influence prescribing practices and which we have classified broadly as patient, prescriber and health system factors. Patient factors included patient expectations, patient load, and patients' health-seeking behaviour. Prescriber factors such as uncertain diagnosis and reliance on clinical judgment; and health system factors such as poor laboratory infrastructure, human resource challenges, and drug quality and availability were important. Prescriber perceived the standard treatment guidelines as basic and felt the need to prescribe outside it. These factors interact in a consultation to influence the outcome, often in favour of presumptive management of patient conditions with antibiotics.
CONCLUSIONS Addressing irrational prescribing practices would require a multi-pronged approach involving all stakeholders especially patients, prescribers, health system administrators, policy makers, government and community. Prescribers often resorted to presumptive treatment in light of inadequate laboratory support and as a means of managing patient load. Interventions targeted at health facilities must, among others, include strengthening laboratory services, and devising strategies to reduce the patient-prescriber ratio.

## 1.7 Bacterial Infections

## 1.7.1 Pneumonia

### O.1.7.1.001

**An update on the causes of morbidity and mortality in sub-Saharan infants**

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INTRODUCTION In 2011, an estimated 7.2 million children died before reaching their firth birth day; 47% of these deaths occurred in Sub-Saharan Africa. Over two-third of all under-five deaths occur within the first year of live. Understanding the common causes of morbidity in this age group may be essential in designing effective disease control and prevention strategies to limit childhood mortality and to support progress toward millennium Development Goal 4.
METHODS A cohort of 1200 healthy infants aged 6–8 weeks were recruited from a semi-urban area (with about 340 000 inhabitants) in The Gambia and followed-up until the age of 12–13 months. The primary outcome was the development of any medical event (e.g. fever, cough, diarrhoea etc) during the course of the study. Infants with such events were seen by dedicated clinicians, where a diagnosis was established and treatment provided. In addition, all infants were seen on a monthly basis for routine assessments and provisions of other primary health care services (e.g. vaccination, vitamin A, dewarming) where appropriate.
RESULTS A total of 39 medical conditions were registered, of which respiratory and diarrhoeal diseases were the commonest causes of ill-health in this age group. Skin conditions such as abscesses, impetigo and dermatitis as well as ophthalmic problems were also important causes of morbidity. The incidence of conditions such as bacterial meningitis, otitis media, malaria and other vaccine preventable diseases was low, suggesting the success of various public health programmes. Only six deaths were registered in this cohort and the causes were bronchopneumonia, gastroenteritis, sepsis and sudden infant death syndrome.
CONCLUSION Sub-Saharan infants suffer from a wide range of medical condition during their first year of life. Our data indicates that early diagnosis of these conditions and provision of adequate treatment is essential in limiting mortality due these diseases.

### O.1.7.1.002

**Potential barriers to healthcare in Malawi for children under 5 years of age with cough and fever: a national household survey**

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INTRODUCTION Adequate access to and utilization of healthcare services is crucial to improve child health in developing countries. However, the rate of obtaining care from a trained healthcare provider remains low in many developing countries; instead, children are often treated at home, by an untrained provider, or not treated at all.
MATERIAL AND METHODS In a national household survey in Malawi, we explored demographic and socioeconomic barriers to healthcare for childhood illnesses and assessed the direct and indirect costs of seeking care. Using a cluster-sample design, we selected 2697 households and interviewed 1669 caretakers of children aged <5 years.
RESULTS Among 2077 children aged <5 years, 504 episodes of cough and fever during the previous 2 weeks were reported. A trained healthcare provider was visited for 48.0% of illness episodes. A multivariate regression model showed that children from the poorest households ($P = 0.02$) and children aged >12 months ($P = 0.02$) were less likely to seek care when ill compared with those living in wealthier households and older children, respectively. Families from rural households spent more time traveling compared with urban households (68.9 vs. 14.1 min; $P < 0.001$). In addition, visiting a trained healthcare provider was associated with longer travel time ($P < 0.001$) and higher direct costs ($P < 0.001$) compared with visiting an untrained provider.
CONCLUSIONS We demonstrated that several barriers to obtain healthcare in Malawi for childhood illnesses exist. Continued efforts to reduce these barriers are needed to narrow the health and healthcare equity gap in Malawi.

## 1.7.4 Other bacterial infections

### O.1.7.4.001

**Yaws prevalence among the Aka in the Republic of Congo, 2012**

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INTRODUCTION Yaws is an endemic treponematosis often seen in isolated, marginalized populations. Early stages primarily affect children, causing self-limited skin and bone lesions. Untreated, after years of latency, 10% will develop disfiguring late lesions. The recent Morges strategy, based on universal treatment with single-dose azithromycin in affected communities, aims to eradicate yaws. Médecins sans Frontières began implementing this strategy among the Aka pygmies in September 2012, with a follow-up campaign in April 2013. We conducted cross-sectional prevalence surveys during both campaigns.
MATERIAL AND METHODS The study took place in the Bétou and Enyellé Districts. At each treatment site, a physician screened Aka <15 years for active yaws. A treponemal rapid diagnostic test (RDT) was performed on children with lesions suspicious for yaws. Children with suspect lesions and positive RDT were considered confirmed cases of active yaws. Prevalence was calculated at district- and village-level.
RESULTS Six thousand two hundred and fifteen children were treated and screened during the first treatment campaign. Four hundred and eighty-five (7.8%) had suspicious lesions; 480 (99.0%) accepted confirmatory testing. Among those tested, 183 (38.1%) were RDT-positive and considered confirmed cases, of whom 107 (58.5%) were boys, and 89 (48.6%) aged 5–9 years. The most common clinical manifestations were papillomata and ulcers. The baseline prevalence was 2.0% in Bétou District and 3.8% in Enyellé District; the spatial distribution of cases was highly heterogeneous. Prevalence was 2.3% in villages accessible by road; in villages accessible only by foot, it was 7.5%. Preliminary results of the second survey show decreased yaws prevalence in the District of Enyellé. Full results will be available in May 2013.
CONCLUSION We show the high burden of yaws in remote regions of Congo, underscoring the logistical challenges that yaws eradication poses. The follow-up survey suggests that the Morges strategy is effective in initially reducing yaws prevalence, but further evaluation is needed.

### O.1.7.4.002

**Towards enhanced management and control of Buruli ulcer disease in Togo 2007–2013: implementation of active case finding and a national reference laboratory**

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Since the early 1990s, more than 1800 suspected cases with Buruli ulcer disease (BUD) have been reported from Togo. From 2007 to 2010, a cooperation between the German Leprosy and Tuberculosis Relief Association and the Department of Infectious Diseases and Tropical Medicine, University Hospital, Munich, allowed for the first time external quality assurance (EQA) for microscopy and PCR analysis of diagnostic samples from suspected BUD cases. Insufficient case finding efficiency, poor performance of local microscopy and turnaround times associated with shipment of PCR samples to Germany however, caused a considerable diagnostic delay and necessitated the implementation of outreach programmes and a national BUD reference laboratory in Togo. To enhance active case finding, outreach programmes were integrated in an existing network for TB and leprosy control through continuous training of health care professionals and large scale 'information, education and communication' campaigns in two BUD-endemic regions in Togo. Suspected BUD patients were referred to health facilities for diagnosis and treatment. Microscopy was conducted at regional level and accompanied by EQA at national and external refer-


 

ence level. A molecular laboratory unit was installed in Togo, and sample pairs were analyzed by IS2404 PCR at national and external reference level. All procedures in the field and laboratories were defined in standard operating procedures. During the study period the rate of BUD patients with non-ulcerative lesions increased from 37% to 50%, the mean duration of disease for ulcerative lesions and the percentage of category III lesions decreased from 182.6 to 82.1 days, and from 30.3% to 19.2% respectively, thus reflecting the impact of outreach programmes accompanied by regular training on case finding efficiency. Case confirmation rates of 50% (microscopy) and 78% (PCR) as well as high inter-laboratory concordance rates (89–94% for microscopy, 96% for PCR) suggest excellent performance of the local reference laboratory.

### O.1.7.4.003

**First isolates of *Leptospira interrogans* from rodents and leptospirosis seroprevalence of human patients with malaria symptoms in Luanda and Huambo provinces (Angola)**

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INTRODUCTION Leptospirosis is the most worldwide distributed zoonosis and the rodents are the main reservoirs of causative agent. In Angola, the diagnostic of the human febrile illness cases is limited and Malaria, the common suspicion.
OBJECTIVES (i) To analyze the rate of *Leptospira* spp from rats and mice by kidney culture and by Polymerase Chain Reaction (PCR); (ii) To know the seroprevalence of antibodies against *L. interrogans* sensu lato (s.l.) in patients with febrile illness and their infection risk exposure.
MATERIAL AND METHODS About 77 small mammals (rats and mice) were trapped and the kidneys were extracted for amplification of *Leptospira* DNA and/or inoculate in selective medium EMJH from the live rodents. Two PCR assays targeting *rrs* (16S) and *hap1* genes, universal and specific for pathogenic leptospires, respectively, were performed. The amplified DNA has been sequenced. For the human patients, was applied a clinical-epidemiological questionnaire and collected sera from 650 patients with fever illness at the health institutions in referred provinces. Serological results were obtained by Microscopic Agglutination Test (MAT).
RESULTS DNA of *Leptospira* spp was amplified from 10/77 (13%) rodent's samples; Two of them were sequenced and showed a homology of 99% for serovar Lai of *L. interrogans*. From 37 rodent's kidneys culture, were obtained 4 (10.8%) *Leptospira* isolates. Among the patients, the data showed high contact with risk factors include the presence of rodents. From the total sera analyzed, was found 88 (14%) samples with specific reactivity: 50 (8%) of them with agglutinins anti-*L. interrogans* (s.l.) for several serovars with titres ≥1:100 (positive) and 38 (6%) with borderline titres.
CONCLUSIONS (i) First isolates of *Leptospira* spp from rodents in Angola; (ii) Evidence for human contact with *L. interrogans;* (iii) This pioneer study reinforces the need for Leptospirosis diagnostic from the Malaria's suspected cases.

## 1.8 Tuberculosis, lack of resources or lack of attention?

### O.1.8.001

**TB in sub-Saharan Africa: present status and immediate challenges**

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Tuberculosis is a chronic infectious disease mainly caused by *M. tuberculosis*. It is one of the major public health problems in the world, causing ill-health among millions of people every year and ranks as 2nd leading cause of death from an infectious disease worldwide, after HIV. Geographically, the burden of TB is highest in Asia and Africa. The African Region with a third of world's population accounts for more than 80% of TB cases in the world and has the highest rates of cases and deaths relative to population. This particular presentation aims at providing highlight on the current TB situation and challenges in Sub-Saharan Africa. Of the 22 TB High Burden Countries in the world, nine are from Africa and account for almost 31% of incident TB cases. Of the 6.2 million TB cases notified in 2011, 24% were from Africa region and quarter of these were from South Africa. Among the 15 countries with the highest estimated TB incidence rates, 13 are in Africa, linked to high rates of HIV co-infection. Almost 80% of TB cases among people living with HIV reside in Africa. Though the TB incidence rates are declining in all WHO's six regions, the Africa region will not be able to halve the number of TB cases by 2015 as other regions like America. Several challenges exist and hampers the African region from achieving the targets and the millennium goals by 2015, these include; TB diagnostics, treatment and care, TB/HIV co-infection, multi-drug resistance (MDR-TB) and prevention (vaccine and preventive therapy). Other challenges are those related to health system; such as shortage of health workers to couple with high number of TB cases and infrastructure. More integrated and innovative approaches are needed to address the TB control challenges in Sub-Saharan Africa.

### O.1.8.002

**Sex, smoking and antiretroviral therapy are predictors of body composition changes during tuberculosis treatment in Mwanza, Tanzania**

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INTRODUCTION Gains in fat mass (FM) and fat-free mass (FFM) during tuberculosis (TB) treatment may determine functional recovery and survival, yet data are scarce. We aimed to assess predictors of FM and FFM changes at the end of 2-months of intensive TB treatment phase in a cohort in Mwanza, Tanzania.
MATERIALS AND METHODS FM and FFM were determined at the start of TB treatment and after the 2-months intensive phase using deuterium dilution technique. Gains in FM and FFM

were determined and predictors assessed using multiple regression analysis.
RESULTS Of 201 patients recruited, 37.8% were females and mean (SD) age was 36.8 (13.1) years, 65.3% (126) had sputum-positive TB, 51.7% (104) were HIV-infected, and 24.4% (49) were current smokers. Among 116 (57.7%) followed-up, mean weight gain was 3.3 kg (95% CI: 2.7; 3.8) and it did not differ by sex (−0.2, −1.4; 1.0). However, compared to females, males had 1.0 (0.4;1.6) kg/m$^2$ lower FM, but 0.7 (0.2, 1.3) kg/m$^2$ higher FFM gain. Current smoking was associated with higher FM (0.7 kg/m$^2$, 0.04, 1.4), but lower FFM (−0.5 kg/m$^2$, −1.2, 0.07) gain. Among HIV-infected patients, antiretroviral therapy (ART) was associated with lower FM gain (−1.2, −2.2, −0.2) kg/m$^2$, but not FFM.
CONCLUSIONS During intensive phase of TB treatment, sex, smoking and ART were predictors of body composition. Larger and longer studies are warranted to further understand predictors of FM and FFM gain at TB treatment completion and beyond to help design interventions to improve treatment outcomes.

### O.1.8.003
**Risks of multidrug and extensively drug-resistant tuberculosis in patients with multiple previous treatments in rural China**

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BACKGROUND Tuberculosis (TB) patients with a history of multiple anti-TB treatments are the 'neglected' patient group to the free anti-TB treatment policy in China. This study aims to understand patients experiences on their previous treatments and to what extent second-line anti-TB drugs had been used in the basic units of TB medical care, and how this might influence the risks of multidrug and extensive drug-resistant TB (M/XDR-TB).
METHODS A cross-sectional study was carried out in 10 county/district TB clinics purposively sampled from five provinces of China. The study participants were TB patients with a history of at least two longer-than-1-month treatment episodes. Face-to-face interviews and drug susceptibility testing (DST) were given to the consented participants.
RESULTS A total of 328 eligible TB patients were recruited, 30.6% of them had more than two prior treatment episodes. The proportion of MDR-TB was 58.2% in the 287 DST completed patients. About 23.8% of the participants had a history of taking second-line drugs, with 5.5% and 14.6% in their 1st and 2nd treatment episode respectively, and more than 77.8% of them were treated in county TB dispensaries where only sputum microscopy was applied in diagnosis. Multivariate analysis found that frequency of previous treatments was significantly associated with the use of second-line drugs ($P < 0.01$), whereas no statistically significant associations were found between the use of second-line drugs and MDR status, age, gender and medical insurance.
CONCLUSIONS Patients with multiple previous treatments are at high risk of M/XDR-TB in China. Use of second-line anti-TB drugs has not been well regulated. The behavior of prescription of second-line drugs mainly depends on the treatment history of patients, rather than drug-resistance profile. DST guided treatment should be assured for the high risk TB patients in order to prevent the epidemics of XDR-TB.

## 1.10 Diarrhoeal Diseases

## 1.10.2 *Cryptosporidium* a public health concern – recent advances in our understanding of the epidemiology

### O.1.10.2.001
**_Giardia lamblia_ infections among patients with diarrhea attending a rural hospital in Ethiopia**

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INTRODUCTION Intestinal parasitic diseases are a huge burden for low-income countries. The aim of this study was to determinate the prevalence of *Giardia lamblia* in patients with diarrhea attending a rural hospital in southern Ethiopia and to analyze their association with age, gender, and other intestinal infestations.
METHODS A retrospective observational study of all stool samples examined for parasite detection in the period 2007–2012 at Gambo Rural Hospital. Stool examination was performed in patients who presented diarrhea.
RESULTS Thirty-two thousand one hundred ninety-one patients with diarrhea were included in this study. Fifty-two percent of the study subjects were female. The median age was 18 (interquartile range [IQR]: 5–30). The overall prevalence of intestinal parasites was 26.5%. The predominant parasite detected was *G. lambia* (15.0%), followed by *Entamoeba histolytica*/dispar (5.4%) and *Ascaris lumbricoides* (5.0%). The predominant mixed infections were *G. lamblia* and *E. histolytica*/dispar co-infections (1.3%), *G. lamblia* and *A. lumbricoides* co-infections (0.5%), and *G. lamblia* and Hymenolepis species (0.02%). The median of age of diarrheal patients with *G. lamblia* was 18 years (IQR: 6–30). *G. lamblia* was the most common intestinal protozoan in all age groups, and there was a significant decrease (from 16.7% in <5 years to 11.9% in >59 years (chi-square test for trend = 77, $P < 0.001$) Significant associations between intestinal parasites, sex, and age from multivariate logistic regression models were analyzed. *G. lamblia* infection showed a significant positive association with *E. histolytica*/dispar [adjusted odds ratio (AOR): 2.18; 95% confidence interval (CI): 1.95–2.45] and a negative association with Taenia sp. (AOR: 0.61; 95% CI: 0.42–0.89) and *A. lumbricoides* (AOR: 0.76; 95% CI: 0.65–0.89).
CONCLUSIONS *Giardia lamblia* was the most prevalent intestinal parasite detected in stool samples in patients with diarrhea.

### O.1.10.2.002
**Cryptosporidiosis in Estonia – a neglected occupational risk**

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No human cases of cryptosporidiosis have been reported in Estonia after year 2000. This is in stark contrast to one of the highest incidences per capita of reported giardiasis in Europe – a parasite which share many transmission routes with Cryptosporidium infections. Cattle are potential reservoirs for human infec-


 

tions with zoonotic Cryptosporidium species such as *C. parvum*. We present the first evidence from Estonia of zoonotic *C. parvum* transmission. One person of a research team fell ill 4 days after sampling calves in 2007 and suffered from stomach cramps, nausea, anorexia, fatigue, muscle aches, fever, and malodorous, watery diarrhoea. Fecal samples collected on days 6 and 14 tested positive for *C.* spp. oocysts using the Ziehl-Neelsen staining technique. The DNA was extracted from the two human samples and from nine cattle samples from different farms suspected as the source of infection. Identification to species level was done by PCR amplification and sequencing of the small subunit ribosomal RNA gene (18S rDNA) locus and the 70-kDa heat shock protein gene (HSP70). Subgenotyping was accomplishing by amplification of the hypervariable glycoprotein (gp) 60 gene. The human sample and one of the calf samples were identical to *C. parvum* sequences in GenBank; and subgenotyping revealed IIaA15G2R1, which has previously been associated with human infections and outbreaks of bovine origin. The person affected consulted a general practitioner during the illness but no diagnostics were attempted. Veterinary students visiting cattle farms in Estonia have previously contracted clinical symptoms consistent with crypotosporidiosis. In some of these cases, students sought medical help in Finland and were diagnosed with cryptosporidiosis; and at least one student was hospitalized. In Estonia, cryptosporidiosis appears to be underdiagnosed and increased awareness is needed.

## O.1.10.2.003

### Causes of acute diarrhea among children younger than 5 years in a rural place in Southern Ethiopia: viral infections

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INTRODUCTION About 1.3 million of death every year due to diarrheal illnesses in the first 5 years of life,most of them in low-income countries.The objective of the study was to know the etiology of diarrhea among children with moderate and severe dehydration in a rural hospital in Southern Ethiopia.
MATERIAL AND METHODS Prospective cross-sectional study conducted September–November 2012 in Gambo Rural Hospital (GRH), Ethiopia. Children under 5 years with moderate or severe acute dehydration were included. Feces specimens were

**Table 1** Children rotavirus-positive and rotavirus-negative.

| | Rotavirus positive ($n$ = 137) No. (%) | Rotavirus negative ($n$ = 177) No. (%) | $P$-value | OR (95% CI) |
|---|---|---|---|---|
| Age months, median (IQR) | 8.0 (6–11) | 9 (6–14.2) | 0.001 | 0.94 (0.91–0.98) |
| Sex | | | | |
| Female | 63 (46.0) | 87 (49.2) | 0.62 | 1 |
| Male | 74 (54.0) | 89 (50.3) | | 1.15 (0.73–1.80) |
| Fever at home | | | | |
| No | 36 (26.3) | 49 (27.7) | 0.52 | 1 |
| Yes | 128 (72.3) | 101 (73.7) | | 1.07 (0.65–1.78) |
| Weakness | | | | |
| No | 6 (4.4) | 2 (1.1) | 0.15 | 1 |
| Yes | 131 (95.6) | 175 (98.9) | | 0.250 (0.05–1.26) |
| Malaise | | | | |
| No | 6 (4.4) | 2 (1.1) | 0.15 | 1 |
| Yes | 131 (95.6) | 175 (98.9) | | 0.25 (0.05–1.26) |
| Cough | | | | |
| No | 103 (75.2) | 89 (50.3) | <0.001 | 1 |
| Yes | 34 (24.8) | 88 (49.7) | | 0.33 (0.21–0.54) |
| Vomiting | | | | |
| No | 8 (5.8) | 29 (16.4) | 0.007 | 1 |
| Yes | 129 (94.2) | 148 (83.6) | | 3.16 (1.39–7.16) |
| Days of diarrhea, median (IQR) | 3 (2–5) | 5 (3–7) | 0.002 | 0.92 (0.88–0.97) |
| Watery diarrhea | | | | |
| No | 0 (0) | 54 (30.5) | <0.001 | |
| Yes | 137 (100) | 123 (47.5) | | NA |
| Blood in feces | | | | |
| No | 134 (97.8) | 135 (76.3) | <0.001 | 1 |
| Yes | 3 (2.2) | 42 (23.7) | | 0.07 (0.22–0.24) |
| Mucus in feces | | | | |
| No | 136 (99.3) | 118 (66.7) | <0.001 | 1 |
| Yes | 1 (0.7) | 59 (33.3) | | 0.02 (0.02–0.18) |
| Pervious treatment | | | | |
| No | 120 (72.3) | 128 (87.6) | <0.001 | 1 |
| Yes | 17 (27.7) | 49 (12.4) | | 2.70 (1.47–4.95) |

IQR, interquartile range; OR, odds ratio; CI, confidence interval.

examined by direct microscopy and antigens of rotavirus where detected by immunocrhormatography.
RESULTS Three hundred and fourteen children were included, 69% was under 12 months. 46.6% of children had rotavirus infection, 6.7% had intestinal parasite infection (IPI), 51.1% had no virus or parasites in feces (probably bacterial infection; PBI). Media of age of children with rotavirus-positive was 9.2 months ($\pm$5.8) ($P = 0.001$), IPI was15.9 $\pm$ 8.4 ($P = 0.002$). Vomiting was in 94.2% of rotavirus infection ($P = 0.004$) and in 76.2% of IPI ($P = 0.08$). Mucus in feces was less common in rotavirus infection (19.1%) ($P < 0.001$), and PBI(5.6%) ($P < 0.001$). Bloody diarrhea was presented in 2.2% of children rotavirus-positive ($P < 0.001$), and IPI was 71.4% ($P < 0.001$). Mean of days with diarrhea was 5.8 $\pm$ 5.9, with rotavirus-infection was 4.2 $\pm$ 5 ($P < 0.001$), with IPI(8.2 $\pm$ 8.1) ($P = 0.05$). All of children with rotavirus infection had watery diarrhea, 47.5% of children with other type of diarrhea ($P < 0.001$). Severe dehydration was presented in rotavirus infection was 72.3% ($P < 0.001$), in PBI was 62.7% ($P < 0.001$) and in IPI 4.8% ($P < 0.001$). Sixty-one percent children were admitted, 88.1% with PBI ($P < 0.001$), 86.1% in rotavirus infection ($P < 0.001$), and 38.1% of IPI ($P = 0.02$). Mortality rate was 1.9%, 4.4% was in rotavirus infection ($P = 0.005$).
CONCLUSIONS Rotavirus infection is an important cause of severe dehydrating diarrhea in young children. Symptoms of diarrhea were different in patients with rotavirus infection, PBI and IPI. Mortality rate was low, but higher in cases of rotavirus infection.

### O.1.10.2.004
**Molecular typing as a tool for Cryptosporidium surveillance and outbreak investigations in Sweden**

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In 2010 and 2011 Sweden experienced two large waterborne outbreaks of Cryptosporidium where it was estimated that over 40 000 persons became infected. These incidences highlighted a need in Sweden for better surveillance of this parasite and in 2012 a 3 year national project was initiated. One of the project goals is to use molecular tools to survey Cryptosporidium species and subtypes that can cause human disease. Local microbiology laboratories are requested to send in fecal samples from all diagnosed human Cryptosporidium cases to the Swedish Institute for Communicable Disease Control for species differentiation and subtyping based on the amplification of the SSU rRNA and the 60 kDa glycoprotein (GP60) genes with subsequent RFLP or sequencing. Seven different species/genotypes have been identified since this study started in October 2012: *C. parvum* ($n = 38$), *C. hominis* ($n = 5$), *C. meleagridis* ($n = 2$), *C. felis* ($n = 1$), *C. canis* (1), *C. viatorum* ($n = 1$) and *Cryptosporidium chipmunk* genotype I ($n = 1$). These data include three outbreaks (two potentially foodborne and one zoonotic) which were all caused by different *C. parvum* GP60 subtypes. Molecular typing is a useful tool, facilitating outbreak investigations and surveillance of Cryptosporidium. This project will give a greater insight into the different species and subtypes causing cryptosporidiosis in Swedish patients. It will also be possible to compare subtypes involved in outbreaks with those present in animal and environmental samples, thereby improving our understanding of Cryptosporidium epidemiology in Sweden.

### O.1.10.2.005
**_Giardia intestinalis_: new strategies to survive a hostile environment**

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INTRODUCTION The microaerophilic protozoon *Giardia intestinalis* is the causative agent of a worldwide intestinal infection, that is a cause of morbidity and mortality especially in infants and elderly. The parasite, although highly vulnerable to $O_2$, preferentially colonizes the fairly $O_2$-rich mucosa of the proximal small intestine. This environment is particularly hostile for a parasite lacking most of the common antioxidant systems (such as superoxide dismutase, catalase, glutathione peroxidase and glutathione itself). Novel genes putatively coding for antioxidant proteins, probably acquired by lateral gene transfer, however, have been recently identified in the parasite. Giardia is indeed the only pathogenic protist as yet identified encoding a flavohemoglobin (flavoHb) and among the very few eukaryotes encoding a superoxide reductase (SOR) and a flavodiiron protein (FDP).
MATERIAL AND METHODS Genes coding for flavoHb, FDP and SOR were cloned and expressed in *E. coli* as recombinant His-tagged proteins, and purified by affinity column. Proteins were functionally characterized both as isolated and in living parasites by high-resolution respirometry, NO-amperometry and time-resolved spectroscopy. Expression in parasitic cells and modulation in stress conditions were assayed by immunoblotting and RT-PCR.
RESULTS We found that the newly identified proteins are expressed in Giardia to protect the parasite from oxidative and nitrosative stress. In particular, FDP is involved in $O_2$ metabolism, reducing it to $H_2O$. FlavoHb is promptly induced by nitrosative stress and efficiently metabolizes NO under aerobic conditions, whereas SOR detoxifies superoxide anion to $H_2O_2$ avoiding $O_2$ release. New data about peroxides metabolism in the parasite will be also presented.
CONCLUSIONS It is suggested that these novel enzymes, by enabling survival of Giardia to oxidative/nitrosative stress in the human intestine, may contribute to parasite pathogenicity, thereby representing potential targets for new therapeutic strategies.

## 1.13 HIV/AIDS

### 1.13.2 HIV – treatment adherence in resource constrained settings

### O.1.13.2.001
**Efavirenz dose reduction in HIV-infected patients**

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First-line treatment with two nucleoside reverse transcriptase inhibitors (NRTIs) plus efavirenz (EFV) 600 mg daily is standard of care in HIV infection. Due to high pharmacokinetic variability and possible relationship with CNS side effects, some patients benefit from EFV dose reduction. We treated 33 patients with two NRTIs plus EFV at reduced doses. EFV plasma levels were


 

measured in blood samples collected 9–16 h after last dose intake. Three groups of patients were studied. Group 1 (16 patients) reduced EFV (due to sleep disturbances) to 400 mg after a mean of 66 months on full dose and when HIV-RNA was <50 copies/ml. Group 2 (seven patients) had a mean 35 months therapy and HIV-RNA <50 copies/ml before EVF reduction to 400 mg by physicians in charge due to sleep disturbances and prior to knowing pharmacokinetic data. Group 3 included 10 naïve patients with a pretreatment mean HIV RNA level of 104 529 copies/ml who started EFV at reduced doses. After 27–109 months (mean 38 months) on reduced efavirenz doses, all but one patients have undetectable HIV-RNA and their CD4 cells continue to be higher than 350/$\mu$L. Although 10 patients (in groups 1 and 2) had below minimum effective concentration efavirenz levels after dose reduction, only one virological failure was observed over an up to 37 months follow-up period. These results confirm previous studies that questioned relationship between plasma levels and efficacy of efavirenz and suggest that long-term maintenance phase of an efavirenz-containing fully suppressive first-line regimen could require lower pharmacological pressure. In conclusion, dose reduction of efavirenz to 400 mg once daily is a feasible therapeutic option that warrants further investigation. The considerable cost savings of reduced efavirenz doses could be very important especially in resource-limited countries.

## 1.14 Vaccines

### 1.14.1 Malaria vaccines

#### O.1.14.1.001

**Immunological lessons learned from testing the malaria vaccine candidate GMZ2 in phase 1 clinical trials**

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INTRODUCTION The malaria vaccine candidate GMZ2 is a hybrid protein consisting of the N-terminal region of the Glutamate-Rich protein fused in frame to the C-terminal region of Merozoite Surface Protein 3 formulated in $Al(OH)_3$. GMZ2 has been tested in three published Phase 1 clinical trials and the GMZ2/alum formulation showed good safety, tolerability and immunogenicity.
MATERIALS AND METHODS Here we present a comparative assessment of the immunological response measured in three Phase 1 trials using serum samples prior to vaccination and 4 weeks after last vaccination. Specific GMZ2 antibody levels, avidity and the biological activity against Plasmodium falciparum are compared.
RESULTS Maximum levels of IgG antibodies were obtained by GMZ2 vaccination independent of ethnicity, time under malaria-exposure, or vaccine dose. Peptide-mapping revealed that antibody responses were mainly directed against epitopes in the GLURP part of GMZ2 and less so against the MSP3 part. Both, avidity and biological activity increased following GMZ2 vaccination and IgG subclass responses identified IgG3 against a peptide derived from MSP3 as the strongest predictor of *in vitro P. falciparum* inhibitory activity. The immunized volunteers elicited vaccine-specific antibodies with inhibitory activity against geographically diverse *P. falciparum* isolates.
CONCLUSIONS These findings suggest that GMZ2 adjuvanted in $Al(OH)_3$ elicits specific and functional antibodies with the capacity to control parasite multiplication.

#### O.1.14.1.002

**Doctors and vampires in Sub-Saharan Africa: ethical challenges in clinical trial research**

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Collecting blood samples from individuals recruited into clinical research projects can be challenging in Sub-Saharan Africa. Strikingly, among the reasons are local rumors of 'blood stealing' or 'blood selling'. Biomedical researchers commonly dismiss such 'rumors' as evidence of the local population's ignorance of trial goals and procedures, and, hence, give them little credence. Nevertheless, such fears can potentially have dire effects on the implementation of research projects and have ethical implications related to cultural sensitivity and consent. We present a step-by-step account of the underlying logic governing patients' fear of providing blood samples in African contexts, drawing from a general literature review and from ethnographic data collected in Gabon. We aim to demonstrate that such fears represent rational attempts at making sense of sickness and health in an unjust and often-incomprehensible world. Furthermore, given the nature of these rumors, we argue that the currently adopted solution to address such concerns -namely, improving the informed consent process by providing additional information – does not suffice to prevent or dispel existing doubts or distrust of clinical research.

#### O.1.14.1.003

**Introduction of malaria vaccine in Nigeria: status and progress update**

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BACKGROUND Malaria is endemic in Nigeria. About 97% of the population is at risk and it accounts for 25% under-5 mortality. Artemisinin-based Combination Therapy and Intermittent Preventive Treatment are current curative and preventive interventions. Clinical trials using malaria vaccine were conducted in Enugu and Jos, Nigeria. The RTS, S/AS01 coadministered with Expanded Programme on Immunization (EPI) vaccines provided modest protection against both clinical and severe malaria in young infants.
METHODS Nigeria officials held Stakeholder and advocacy meetings with facilitators from MVI PATH in 2011 and 2012. Key informants were interviewed about national policy decision making processes on adopting new malaria control interventions and new vaccines in Nigeria and the readiness of the health system in adopting the candidate vaccine. The results were analyzed by content analysis.
RESULTS Progress is being made with adoption of malaria vaccine in Nigeria. There was no National Immunization Technical Advisory Group/Committee for all vaccine preventable diseases in Nigeria, and no standard documented guideline for decision making process for the adoption of new vaccines. The existing public-private partnership for the adoption of Human Papilloma Virus vaccine and Expanded Programme on Immunization in Nigeria was proposed for Malaria vaccine. Challenges foreseen with the decision-making process include large population size, weak health system, resistance to change by health staff, inadequate trained staff, high cost of

implementation, budgetary deficits, inadequate cold chain and storage supply. Recommendations

A proposal presentation would be made to the Technical Working Group on Malaria and relevant stakeholders.Funding will be harnessed from World Health Organization and Global Alliance for Vaccine and Immunization. A proper cost benefit analysis would be done to ascertain cost-effectiveness. High level advocacy will be carried out at all levels.

### 1.14.2 Tuberculosis vaccines

#### O.1.14.2.001

**The effect on hospital admissions of providing BCG vaccine at birth to low-birth-weight infants: randomised trial in Guinea-Bissau**

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OBJECTIVE To examine the effect of early BCG vaccination to low-birth-weight (LBW) infants on the risk and causes of neonatal and infant hospital admissions. Design: Study of a secondary outcome within a randomised controlled trial (RCT) of BCG-at-birth (intervention) versus delayed BCG (current practice).

MATERIALS AND METHODS From 2004 to 2008 we recruited 2320 LBW children for the RCT. The children were examined, randomised and if randomised to BCG-at-birth vaccinated with BCG (standard 0.05 ml dose of Statens Serum Institut (SSI) BCG by intra-dermal injection) by our team at the maternity ward at the national hospital Simão Mendes and at several health centres. Data on hospital admissions including duration, cause and outcome were collected at the paediatric ward, Simão Mendes and at home follow-up visits during the first year of life. Setting: Urban Bissau, Guinea-Bissau. Main outcome measures: We studied the effect of BCG-at-birth on neonatal (28 days) and infant (1 year) hospitalisation incidence in Cox regression models providing incidence rate ratios (IRR). We also calculated IRRs for major disease groups, including malaria, pneumonia, diarrheal diseases and septicaemia. All analyses were stratified by sex and vitamin A status.

RESULTS The risk of neonatal hospitalisation was not significantly lower in the group receiving BCG-at-birth compared with BCG-unvaccinated children, the neonatal IRR being 0.73 (CI: 0.46–1.17) ($P = 0.19$). By 1 year of age, the IRR was 0.99 (CI: 0.78–1.24). Further analyses of multiple hospital admission events and stratification on sex, vitamin A status and inclusion of maternally reported admissions will be performed and presented at the meeting.

CONCLUSION In the preliminary analysis, we found a non-significant tendency for reduced risk of neonatal hospital admissions. Further analyses are planned to shed further light on the effects of providing BCG at birth to LBW children on the risk of overall and disease-specific hospital admissions.

## 1.15 Drug quality

### 1.15.1 Counterfeited drugs in developing countries

#### O.1.15.1.001

**Quality assurance of health care and risk management related to conterfeit drugs in Niger**

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INTRODUCTION While quality assurance (QA) in health care in developed countries, taking into account the standards of best performance with safety and standard of services, QA in health care in developing countries has very different aspects in relation to their very different health priorities, but also to a doubtful environment of the drugs, very inappropriate and sometimes even dangerous for patients life.

MATERIAL AND METHODS It an overview of our national QA practice focused on the treatment of some diseases with high mortality rate from village health center to referral national teaching hospital with an emphasis on training facilities by using Telemedicine. On other side, we make an analytic review of the counterfeit medicines and we discuss the medical problems which occur.

RESULTS Thus, QA is mainly oriented to strategies for reducing neonatal mortality, infant, child and maternal mortality and improving the integrated management of childhood illness, the essential obstetric and neonatal diseases taking into account the QA in healthcare environment, the prevention of nosocomial infections and hospital hygiene, training of health care providers, at all levels of the health pyramid. But a better dispensation of quality drugs is essential to quality care, especially for the developing countries because nowadays they are invaded by drugs from different backgrounds sometimes pirate laboratories which are deficiently dosed often exposing to multiple bacterial resistance, various side effects due to the drugs sold without control by 'hawkers'' and' non-medical or none pharmaceutical traders, which serious endanger the lives of patients.

CONCLUSION As QA in these developing countries need to develop strategies to fight against false drugs and their parallel markets particularly in Sub-Saharan Africa.

# Track 2: Reproductive and Child Health

## 2.2 Child health

#### O.2.2.001

**HIV prevalence among children born to HIV-positive mothers followed in prenatal consultation for prevention mother to child transmission at Buyenzi Communal Medical Centre: retrospective study of 774 children screened January 2008–December 2010**

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INTRODUCTION This was a retrospective study on the prevalence of HIV among children born to HIV positive mothers followed in PNC (Pre-Natal Consultation) for PMTCT. The aim of our study was to observe whether it was possible to eradicate HIV transmission from mother to child in Burundi. To do this, we evaluated the effectiveness of the national PMTCT protocol in reducing the MTCT by the number of HIV infected children

born to HIV followed for PMTCT and analysis of factors that would influence MTCT.
MATERIAL AND METHODS Our study was conducted at Buyenzi CMC-PMTCT site located in Bujumbura Town-and involving infants screened by PCR test from January 2008 to December 2010. We drew up a survey form on the basis of the national PMTCT protocol and objectives of our study.
RESULTS Seven hundred and seventy-four children born to 763 mothers met the criteria of our study. Of these, 11 were infected with a MTCT rate of 1.4%. Some factors were associated with MTCT: low birth weight ($P = 0.01$), knowledge of partner's serostatus ($P = 0.02$) and prematurity ($P = 0.02$).
CONCLUSION The PMTCT in Burundi is effective in reducing HIV transmission from mother to child. It is nearly impossible to eradicate MTCT as long as all pregnant women do not understand the need to get tested and get the required viral load during and after pregnancy if infected.
KEYWORDS HIV-AIDS, PMTCT, prevalence, children, pregnant women, ARV, Burundi.

## 2.4 Medicines for children

### O.2.4.001
**Combating barriers to epilepsy treatment in Western Uganda**

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INTRODUCTION Epilepsy affects three times as many people in Sub-Saharan African countries than in western countries (1). We know from previous studies in Uganda that even with free services, the distance needed to travel to access them is often prohibitive to the people most in need (2). Epilepsy carries a huge stigma for the whole family with many children hidden away to avoid embarrassment or social isolation. Hospital epilepsy clinics in Western Uganda are poorly attended and there is low uptake of free medication and education.
METHODS After assessing attendance and follow-up in the hospital setting of children with epilepsy we offered medical epilepsy clinics outside the hospital. Following this we set up satellite clinics, run by a clinical officer with extra training in epilepsy and funded by a UK charity, in local villages to find the optimum method of treating epilepsy in this predominantly rural area of Uganda.
RESULTS In hospital treatment (free, drop-in/weekly): one child per month with the majority not attending follow-up at the appropriate time. Outside hospital clinics (free for patient, cost to run: $20 per clinic, weekly): two children per month with nearly 100% follow-up. Satellite clinics covering four villiages (free for patient, cost to run: $44 per clinic, monthly): 15–56 children per clinic with 70% attending for follow-up.
CONCLUSION Satellite clinics help to remove the barriers to health care but depend on local people to continue education and encourage follow-up. The clinics provided a forum for children affected by epilepsy and their families to interact and this was noted to be of great help.

## 2.5 Global burden of prenatal morbidity and mortality

## Improving neonatal outcome

### O.2.5.001
**Newborn care in three selected South East Asian countries: a comprehensive needs assessment**

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BACKGROUND As observed globally, decrease of neonatal mortality between 1990 and 2010 in the assessed countries – Indonesia, Lao PDR and the Philippines – was slower than decline of under-5 and infant mortality. As a result the proportion of under-5 deaths due to neonatal mortality increased which urges the need for focus on newborn care. Findings of the comprehensive needs assessment, conducted from November 2012 to March 2013 on behalf of UNICEF EAPRO, will inform country level strategies, work-plans and partnerships for accelerating reduction of neonatal mortality and morbidity.
METHODS An in-depth study of newborn health policies, services and care in the three countries through desk review, stakeholder mapping, key informants interviews and health facility visits was followed by analysis of data found using a 'strengths, weaknesses, opportunities, threats' analysis, an equity analysis and the Save the Children's Scale-up Readiness Benchmarks tool to assess current newborn health situation and country's readiness for scaling up newborn care. Based on the findings, recommendations were formulated.
RESULTS Main findings are: (i) comprehensive newborn policies in line with international standards exist although implementation remains poor, (ii) quality of newborn care is generally substandard, (iii) access to skilled providers is limited, (iv) health sector decentralisation brought opportunities and threats for newborn care, (v) fragmentation across several MoH departments hampers coordination and implementation of newborn care, (vi) socio-economic and demographic inequities in newborn care are considerable, and (vii) regulation and cooperation with the private sector is lacking.
CONCLUSION Similar challenges for newborn care are identified in assessed countries and show need to improve access to quality newborn care. Main opportunities identified to address this need include: strengthening newborn pre-service training, providing supportive supervision and strengthening leadership and skills of mid-level health management to enhance newborn care. Interventions to minimize socio-economic and demographic inequities are urgently needed.

### O.2.5.002
**Delayed cord clamping in a cohort of South African infants with presumed suboptimal intra uterine growth**

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INTRODUCTION Delayed cord clamping (DCC, clamping the umbilical cord 2–3 min after birth) increases haemoglobin (Hb) levels in newborns and reduces iron deficiency risk in both preterm and term infants. It is recommended as costless iron

boost to optimize brain development in early infancy. In term infants with a normal bodyweight the positive effect on their iron status lasts up to 4–6 months. We aimed to evaluate the long-term effects of DCC in infants with suboptimal intrauterine growth born in a resource-poor setting.
METHODS Women in labour with a presumed low birthweight baby (intrapartum symphysal-fundal height <34 cm) were randomized for early cord clamping (ECC, within 60 s) or DCC. Infant Hb-levels and weight were measured at birth, after 24 h and at the age of 2 months. Potential short-term side effects including hyperbilirubinaemia and hyperviscosity were also studied.
RESULTS We included 104 mother-infant pairs, of which 39% actually had a birthweight <2500 g. A total of 73 mother-infant pairs returned for follow-up (70%). There were no differences in baseline characteristics between returnees and defaulters. Twenty-four-hour after birth infant Hb was significantly higher in the DCC-group (18.0 g/dl vs. 16.8 g/dl, $P = 0.006$), implying successful placental transfusion. This effect did not sustain until the follow-up visit at the age of 2 months (Hb 9.9 g/dl vs. 9.8 g/dl, $P = 0.60$). Despite higher Hb-levels in the DCC group 24 h after birth, hyperbilirubinemia and hyperviscosity were not observed. At 2 months the infants in the DCC-group had better weight gain from baseline compared to the ECC-group (2.2 kg vs. 1.9 kg, $P = 0.058$).
CONCLUSION In this South-African cohort of newborns with a subnormal birthweight the effect of DCC on iron status was not detectable anymore 2 months after birth. Weight gain, however, tended to be more favourable in the DCC-group.

## O.2.5.003

### Prospective audit study of neonatal deaths in a pediatric hospital in Vietnam

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INTRODUCTION Neonatal mortality (¡Ü 28 days) comprises more than half of child mortality in Vietnam. The vast majority presumably dies in health care facilities, but data on neonatal hospital mortality is scarce.
METHODS Prospective audit study of all neonatal deaths and withdrawal-of-life-sustaining-treatment (WLST) in a Vietnamese tertiary pediatric hospital in a 12 month period in 2009¯C2010. The medical files were reviewed classifying: prognosis at arrival, discharge outcome, cause of death/expected death according to two classification systems, and potentially avoidable risk factors during the hospital stay.
RESULTS Among 5763 neonates admitted, 235 in-hospital deaths and another 67 WLST were included. According to both classifications, the major causes were congenital malformations, prematurity and severe infections. Among 85% (60/71) of neonates with a relatively good arrival prognosis, 6 potentially avoidable risk factors were identified with relation to danger signs response, internal transfers, nosocomial infections, sepsis management, access to usual of equipment/staff, and family perception.
CONCLUSION Among 302 neonatal deaths or WLST, the major causes were congenital malformations, prematurity and severe infections. Among the neonates with relatively good prognosis at arrival, 6 potentially avoidable risk factors could be addressed without implementation of new technologies or major organizational changes.

**Table 1** Avoidable risk factors among neonates with relatively good prognosis at admission ($n = 71$), $n$ (%).

| | |
|---|---|
| Dealyed recognition and/or response to danger signs | 30 (42) |
| Suboptimal internal transfers | 26 (37) |
| Family perception | 12 (17) |
| Nosocomiel infections | 24 (34) |
| Sub optimal sepsis management | 17 (24) |
| Shortage of equipment/staff (surgeon) usually available | 18 (25) |

Relatively good prognosis at arrival defined as >50% chance of survival and normal development

## O.2.5.004

### Determinants of thymic growth among normal and low birth weight newborns in Guinea-Bissau

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BACKGROUND In Guinea-Bissau having a small thymus gland in infancy is associated with increased mortality. Therefore it is important to determine factors which influence thymic growth; infectious and nutritional factors are known to be important.
OBJECTIVE We aimed to study determinants of thymic growth in two cohorts of 305 normal birth weight (NBW) and 382 low birth weight (LBW) newborns.
METHODS Thymic size was measured with ultrasound after birth and again after 4 weeks. Thymic index (TI) and thymus/weight index (TWI) after 4 weeks were used as outcome measures. Determinants were collected from hospital records and interviews with the mother. Data were analyzed in crude and adjusted linear regression models, providing geometric mean ratios (GMR) with 95% confidence intervals. All estimates were adjusted for thymic size at enrollment.
RESULTS The mean increase in absolute thymic size (TI) was 6.6 for NBW infants and 5.9 for LBW infants, $P = 0.06$, while the mean increase in relative thymic size (TIW) was 0.5 and 0.8 respectively, $P = 0.002$. In the adjusted model hospitalization during follow-up was associated with decreased TI after 4 weeks in both cohorts (GMR, NBW infants: 0.81 (0.66–1.00), LBW infants: 0.56 (0.45–0.70)) as well as a decreased TWI among LBW infants (GMR: 0.69 (0.57–0.82)). Among NBW infants males had an increased TI and TWI (GMR for TI: 1.09 (1.03–1.17)) compared to females. Among LBW infants maternal HIV was associated with decreased TI and TWI (GMR for TI: 0.71 (0.57–0.89). Other determinants of increased thymic growth were high maternal age (NBW infants) and having electricity in the house (LBW infants).
CONCLUSIONS Overall LBW infants had increased thymic growth relative to their weight after birth compared to NBW. Hospitalization, maternal HIV, sex of the infant and some socioeconomic determinants influenced thymic growth after birth.


 

### O.2.5.005

**Is neonatal TSH a useful indicator of iodine deficiency in Lubumbashi?**

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BACKGROUND Maternal iodine deficiency can induce increased serum thyrotropin (TSH) in new-borns. Consequently, neonatal TSH has been proposed as a useful indicator of thyroid function in countries where salt iodination programme has been initiated. In a very recent study, pregnant women from Lubumbashi in Democratic Republic of the Congo were found to be moderately iodine deficient. The aim of our study was therefore to assess thyroid function in new-borns by evaluating neonatal TSH and to compare results with maternal iodine intake and thyroid function.
METHODS 75 neonates born in maternities from urban, semi-urban and rural areas of Lubumbashi were screened for congenital hypothyroidism. We measured TSH in heel-prick blood spot collected on absorbed cards at the day 3 after birth, as recommended by the World Health Organisation. We also measured serum TSH and urinary iodine concentration in mothers. We used 5 mIU/l as cut-off for the neonates and 3.6 mIU/l for mothers.
RESULTS The overall median of TSH was 5.7 [IQR, 3.9–8.5] mIU/l in the neonates, and 2.8 [IQR, 1.8–3.7] mIU/l in mothers. 63% of new-borns had TSH >5 mIU/l, and 11% had TSH >10 mIU/l. Proportion of mothers with TSH >3.6 mIU/l was 27%. Maternal and neonatal TSH were not correlated ($r = 0.06$, $P = 0.61$). Neonatal TSH was not associated with iodine intake of mothers, but proportion of neonates with increased TSH was higher in the rural area, where maternal iodine intake was the poorest.
CONCLUSION Our results suggest severe iodine deficiency in Lubumbashi. However, cut-off of neonatal TSH could be re-evaluated to be a more sensitive indicator of iodine nutritional status.

### O.2.5.006

**Reducing neonatal mortality in Western Uganda**

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According to UNICEF, 99% of maternal and newborn mortality occurs in the developing world. In Uganda, the neonatal mortality is 26 deaths/1000 live births (1), compared to a neonatal mortality rate of 2.9 deaths/1000 live births in the UK (2). Other than the psychological effects of losing a baby, there are financial and social implications for the family and community. Although the vast majority of Ugandan people live in a rural setting, neonatal specialist care outside of urban areas is sparse. Kagando Hospital is a resource poor rural hospital in Western Uganda, which, despite a rapidly growing population, has not previously had specialist neonatal care. Our aim was to introduce basic, affordable guidelines to improve the neonatal care at Kagando Hospital. Working with local clinicians and nurses, we introduced guidelines focusing on three main areas:
1 infection control (isolating infective vs premature babies)
2 management of sepsis (early antibiotic therapy)
3 fluids and nutrition (feeding assessments and nasogastric tubes)

We examined the numbers of deaths in the unit before and after intervention, and gauged confidence levels in the wider hospital setting when dealing with neonates. Focusing on babies of between birth and 3 months of age, of any gestation, mortality fell by 56–83% over a three month period. Accuracy was affected by record keeping. Subjectively, post implentation, the confidence level throughout the hospital when dealing with neonates was improved. It is possible to reduce neonatal mortality in the hospital setting in developing countries with minimal expenditure. With clear, simple to follow, realistic and affordable guidelines, the medical staff and the wider community can grow in confidence when dealing with a sick baby. Our interventions and resulting community and international confidence led to increased funding for neonatal care at Kagando Hospital, facilitating a move to a new, purpose built unit.

# Track 3: Non-Communicable Diseases

## 3.2.1 The double burden of communicable and non-communicable diseases

### O.3.2.1.001

**Diabetes is common among ART-naïve HIV positive patients due to impaired beta-cell function**

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OBJECTIVES To assess the prevalence of prediabetes and diabetes among ART naïve HIV positive patients eligible to commence ART.
DESIGN AND METHODS A cross-sectional study in HIV positive patients commencing ART in Jimma, Ethiopia, and HIV negative controls. Plasma glucose and insulin levels, fasting and during oral glucose tolerance test (OGTT), and glycated haemoglobin (HbA1c) were measured, and homeostatic model assessment (HOMA) was calculated to quantify insulin resistance and beta-cell function. A diabetes diagnosis was defined by either HbA1c≥; 6.5% (48 mmol/mol) or from the OGTT (WHO 1999).
RESULTS Among 331 HIV positive patients, the mean (SD) age was 32.9 years (8.8) and 66.9% were females. Based on OGTT, 35.0% had impaired glycaemia, including 6.7% with diabetes. Using HbA1c, 8.5% were diagnosed with diabetes. However, the two methods did not identify the same individuals with diabetes, and in total, 14.3% had diabetes based on at least one of the tests. Among 90 HIV negative controls, 1 (1.1%) had diabetes based on HbA1c. Among HIV patients, prediabetes and diabetes did not seem associated with insulin resistance, however, both prediabetes (eB 0.54, 95% CI 0.41; 0.72, $P < 0.001$) and diabetes (eB 0.34, 95% CI 0.19; 0.58, $P < 0.001$) were associated with a major reduction in beta cell function.
CONCLUSION The prevalence of prediabetes and diabetes was surprisingly high among ART-naïve HIV positive patients, while diabetes was almost non-existing among HIV negative controls. Since diabetes was not associated with insulin resistance, but with reduced beta cell function, the hyperglycaemia may be associated with the HIV infection.

## 3.3 Malnutrition (Malnutrition and infection)

### 3.3.1 Acute malnutrition: short and long-term consequences

#### O.3.3.1.001

**Predictors of oedema among children hospitalized with severe acute malnutrition to Jimma University Hospital, Ethiopia: a cross sectional study**

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INTRODUCTION There are two main clinical manifestations of SAM, i.e. oedematous and non-oedematous. However, which factors lead to oedema and the mechanisms behind have been discussed extensively, but remains unknown. The aim of this study was to identify predictors of oedema in children with SAM.
METHODS Children 0.5–14 years of age with SAM (MUAC <11.0 cm or weight-for-height <70% of median and/or nutritional oedema) admitted to the nutrition unit were included. Information on infections before and during admission was collected together with anthropometry. Predictors of oedema was analysed separately for younger (<60 months) and older children (≥60 months).
RESULTS Three hundred and fifty-one children were recruited (median age: 36 months (interquartile range 24–60); 43.3% females). Oedema was detected in 61.1%. The prevalence of oedema increased with age, peaked at 37–59 months (75%) and declined thereafter. Infection was more common in the younger group (33% vs. 8.9%, $P < 0.001$) and in this group children with oedema had less infections (25.2% vs. 45.1%, $P = 0.001$). In the older group the prevalence of infections was not different between oedematous and non-oedematous children (5.5% v. 14.3%, $P = 0.17$). In the younger group oedema was less common in children with TB (OR = 0.20, 95% CI: 0.06, 0.70) or diarrhea (OR = 0.40, 95% CI: 0.21, 0.73).
CONCLUSION The proportion of oedema in SAM peaked at 3–5 years of age, and the prevalence of infection was lower among children with oedema. The data suggest that oedema result from the infection-immunity interaction, which in turn could be influenced by age of the child but more studies are needed to confirm this.

#### O.3.3.1.002

**Thymus size in children recovering from severe acute malnutrition – preliminary data**

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INTRODUCTION Malnutrition is known to cause higher susceptibility to infectious disease. A possible mechanism for this immune deficiency may be that children with malnutrition have thymic atrophy1. This study aimed to determine predictors of thymic size in children with severe acute malnutrition, and during their nutritional rehabilitation.
MATERIALS AND METHODS Thymus size was measured by ultrasound in 92 children admitted with severe acute malnutrition for in-patient treatment at admission, at discharge, and at follow-up 8 weeks after admission. For comparison, the thymus size was measured in 20 well-nourished children. We planned to measure the Thymic Index, by multiplying the area of the largest thymic lobe by the transverse diameter 2.
RESULTS Severe atrophy of the thymus was present in almost all malnourished children at admission. In some children, the atrophy was so severe that the thymus could not be visualized at all. In the remaining children it was impossible to obtain a transverse image of the thymus. Therefore, the method was modified to only measure the area of the largest lobe. Upon nutritional rehabilitation thymus size increased, although not to values of well-nourished children.
CONCLUSION Children with severe acute malnutrition had severe thymic atrophy. The method used to measure thymic size must be modified in these children. We propose to use the area of the largest lobe, as previously used in children with malnutrition1. Using this method, thymic size increased during nutritional rehabilitation, but not to values of well-nourished children.

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#### O.3.3.1.003

**Undernutrition causes cardiac dysfunction in a piglet model**

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INTRODUCTION Acute undernutrition in young children is widespread, and causes considerable mortality. Studies indicate that cardiac function is affected, but clinical significance is unclear, partly due to lack of good biochemical markers. Based on an experimental piglet model, we aimed to investigate the effect of undernutrition on cardiac function and biochemical markers.
METHODS Four-week old piglets were given *ad libitum* access to either low-nutrient diet consisting of pure maize flour (MAIZE, $n = 12$) or control diet (CON, $n = 12$) for 7 weeks. Temporal changes in plasma levels of pro-atrial natriuretic peptide (proANP) and troponin T (TnT) were measured as markers of cardiovascular disease. Echocardiography was performed on anesthetized pigs at week 7 and cardiac tissue was subsequently collected for analysis of Na/K ATPase density. For comparison, echocardiography and Na/K ATPase density analysis were also undertaken on a reference group consisting of younger pigs without undernutrition but with a body weight similar to maize-fed pigs.
RESULTS Body weight was lower in MAIZE relative to CON pigs (−72%, $P < 0.001$). There was an initial decline in proANP


 

for both MAIZE and CON pigs during the first 3–4 weeks, then a marked increase in MAIZE pigs at 5–7 weeks, relative to CON pigs ($P < 0.05$). Likewise, mean TNT tended to be higher in MAIZE ($P = 0.07$) suggesting myocardial damage. Echocardiography, as indicated by the myocardial performance index, showed left ventricle dysfunction in MAIZE relative to both weight- and age-matched control pigs. Heart to body weight ratio was similar between groups but the heart had a flabby appearance in the MAIZE group. Myocardial Na/K ATPase levels were 50% higher in MAIZE versus age-matched control pigs ($P < 0.01$).
CONCLUSIONS Undernutrition in a piglet model causes cardiac remodeling. Assessment of cardiac function is important in pediatric patients with undernutrition and proANP may be a relevant plasma biomarker of cardiac disease in undernutrition.

## 3.3.2 Complementary feeding: prevention of malnutrition

### O.3.3.2.001

**Body composition in relation to micronutrient status in Cambodian infants: the Winfood Project**

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BACKGROUND AND OBJECTIVES Malnutrition in infancy and early childhood is responsible for over 3 million child deaths yearly. Moreover, malnutrition in early childhood predisposes to a higher risk for non-communicable diseases such as obesity and cardio-vascular diseases later in life through metabolic alterations which are still not fully understood. Rapid growth during the first 2 years of life and fat mass at 2 years of age are strong predictors for later obesity. We investigated body composition in early childhood in relation to micronutrient status.
METHODS Anthropometry (weight, height, MUAC, skinfolds), body composition (deuterium dilution) and micronutrient status (iron, zinc, vitamin A) were measured at 6 month and 15 month of age. The infants participated in the WinFood project, which had as main objective to assess the effectiveness of fortified complementary foods (FCF) in improving health and growth of Cambodian infants.
RESULTS From 269 Cambodian infants data on body composition and micronutrient status were available for both time points. Lean body mass increased with $1.96 \pm 0.59$ kg, whereas the percentage of body fat decreased from 21.7% to 14.9% over the study period ($P < 0.001$). At 6 and 15 month of age, body fat was strongly correlated to ponderal growth (WHZ, $P < 0.01$) but not to length growth (HAZ) or gender. Vitamin A and zinc status were not related to fat mass. Iron status at 6 and 15 months of age significantly correlated with body composition, with infants with no iron stores at endpoint having a higher fat mass (14.0% vs. 15.5%, $P = 0.02$).
CONCLUSION Iron status, but not vitamin A or zinc status, was related to body composition in Cambodian infants. FCF aimed at improving micronutrient status in early childhood may have long term health benefits.
KEYWORDS body composition, iron, infants, obesity.

### O.3.3.2.002

**Effects of animal-source foods and micronutrient-fortification complementary foods on body composition, linear growth, iron status – the WinFood project in Cambodia**

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INTRODUCTION The nutritional quality of CF in developing countries is often insufficient to sustain optimal growth. The Winfood project evaluated the efficacy of two new, processed rice-based CF with local ASF in Cambodia: non-fortified 'WinFood' (WF) with 14% by dry-weight ASF from small-sized fish (Esomus longimanus and Paralaubuca typus) and edible spiders (Haplopelma sp.); an adjusted 'lite' WinFood (WF-L) with 10% by dry-weight ASF from small-sized fish of mixed species, and fortified with mineral/vitamins. The products were precooked by extrusion. The WF-products were compared with two standard products from World Food Programme: Corn-Soy-Blend (CSB+) and CSB++ (8% by dry-weight skimmed-milk powder), in a single-blinded randomized trial.
METHODS Four hundred and nineteen Cambodian infants at age 6 months were randomized to daily rations of one of the four products for 9 months. BC (deuterium dilution) and iron status (serum ferritin and hemoglobin) were measured before and after intervention and anthropometry (knee-heel-length, length, weight, MUAC, head circumference and skinfolds) monthly. Data were analyzed by intention-to-treat.
RESULTS Among 358 children completing the study, no significant difference in BC between the groups where found, but knee-heel length increments differed ($P = 0.046$: WF-L: 3.6 cm, CSB++: 3.6 cm, WF: 3.5 cm, CSB+: 3.4 cm), suggesting that micronutrient-fortified products with 8–10% ASF (CSB++ and WF-L) promoted better linear growth than products without fortification or ASF. Knee-heel and total length increment was significantly higher in the highest food compliance quartile compared to the lowest, across foodgroups. There were no differences in ferritin and hemoglobin concentration. There was higher prevalence of anemic children in the WF group.
CONCLUSION Products with ASF (milk or small fish) and micronutrient premix resulted in slightly better linear growth. Small fish is a cheap ASF with high potential to improve locally produced industrially processed CF.

### O.3.3.2.004

**Early invitation to prenatal food combined with multiple micronutrients is cost-effective compared to iron-folic acid supplementations: results from MINIMat trial**

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MATERIALS AND METHODS Outcome data (under-five mortality rate) came from MINIMat trial (Maternal and Infant Nutrition Interventions, Matlab, Bangladesh), Health and Demographic Surveillance System of International Centre for Diarrhoeal Disease Research, Bangladesh, and published reports. In MINIMat, women were randomized to early (E, around 9 weeks of pregnancy) or usual invitation (U, around 17 weeks) to food supplementation and daily doses of 30 mg iron plus 400 $\mu$g of folic acid (Fe30F), or 60 mg iron plus 400 $\mu$g of folic acid (Fe60F), or MMS. Costing data came mostly from a published study. In MINIMat, EMMS significantly reduced under-five mortality compared to UFe60F. Since UFe60F is currently not in practice, we presented incremental CE ratios for incrementing UFe60F to EMMS and iron-folic acid supplementation to EMMS.
RESULTS Incrementing UFe60F to EMMS averted one extra death @ US $ 659 and incrementing iron-folic acid supplementation to EMMS averted on extra death @ of US $ 2070 to US $ 2218. These comparisons generated one extra life year @ of US $ 22, and US $ 70 to US $ 74, respectively.
CONCLUSIONS incrementing iron-folic acid supplementation to EMMS seems worthwhile from both health economic and public health standpoints.

# Track 4: Health Systems and Resources

## 4.2 Health financing

### O.4.2.001

**Caretakers' willingness to pay for referral in a community case management program in Uganda**

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INTRODUCTION Malaria, pneumonia, diarrhoea and neonatal conditions are major childhood killers. Implementation of integrated community case management (iCCM) for the conditions is advised in low income countries.
OBJECTIVE To assess willingness to pay (WTP) for referral among caregivers of children referred by CHWs to higher level facilities (HFs).
METHODS Case series study of caregivers of referred children 0–59 months from randomly selected villages in four Mid-western Uganda districts. An interviewer administered questionnaire assessed WTP for referral using the bidding method followed by an open ended question for maximum WTP. Factors associated with WTP were explored through a multinomial regression model.
RESULTS Amongst the referrals 30% (60/203) were due to danger signs, 36% drug unavailability and 43%, fever with a negative malaria test. The majority (92%) were referred to a public HF, 3% private facility and 5% to another CHW. About half 54% did not complete referral due to long distance to the HFs 21%; inaccessible HF at night or weekends 25% and child improvement at home 28%. Upon referral, 31% caregivers opted for a clinic, 26% treatment at home, 19% drug shops and 10% another CHW. Referral completion was 2.8 times higher among children with danger signs relative to those without such signs ($P = 0.004$), or those receiving pre-referral treatment ($P < 0.001$). The median WTP was $ 8 range ($0.5–$15). Most caregivers (60%) perceived their stated WTP as unaffordable by the community's majority. Caregiver's level of education was associated with higher WTP values. Preferred referral sites for stated WTP were public hospitals (30%) and level III private HFs (25%) believed to have expert care and good customer care respectively.
CONCLUSION It is not WTP that is mainly affecting referral completion in iCCM but other barriers in access to higher level facilities which need to be addressed.

### O.4.2.002

**A community-based targeting approach of the poorest households: validity in the context of health insurance in Burkina Faso**

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INTRODUCTION There is a strong political movement growing for developing mechanisms that systematically reduce financial barriers to health services for targeted populations. In Burkina Faso there are two schools of thought on this policy question, one that pushes for free health care for pregnant women and children under 5, another that pushes for exemptions for only the most vulnerable populations (indigents). The difficulty that has already been identified in Burkina Faso is the methodology of identifying the most vulnerable populations. Various approaches have been tested in Burkina Faso, including Community Wealth Ranking (CWR) in the context of community-based health insurance in Nouna health district. While CWR has been found to be participatory and transparent, the validity of the method for the targeting of the poorest needs to be confirmed.
MATERIAL AND METHODS An indigent-selection process is judged effective if it minimizes inclusion biases and exclusion biases. The study compares the levels of poverty and of vulnerability of indigents with the general population of Nouna Health and Demographic Surveillance System. A sample of 566 households among the one selected by the CWR has been interviewed and compared to the 10 213 households of the HDSS.
RESULTS Analyzing the households' expenditures, and using the level of 108 454 CFA we were able to estimate that 75% of selected households were poor (79.8 urban/70% rural). We moreover observed differences between the households who enrolled and the one who did not enrolled to health insurance in term of assets and habitat ($P < 0.05$).
CONCLUSIONS The community-based process minimized inclusion biases, as the households selected were poorer and more vulnerable than the rest of the population. However, much research is still needed to define the modalities of such a system, assess its feasibility, and then measure its effectiveness.


 

### O.4.2.003
**Who gets health insurance coverage in India? New findings from nation-wide survey**

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How far the coverage of health insurance available to Indians, both in rural and urban areas? Who can afford to pay for health insurance coverage? This study examines health insurance scenario of India by analyzing the trends and patterns and household characteristics of health insurance policy holders. The study utilized available data from the latest rounds of two nationally representative surveys DLHS (2007–2008) and NFHS (2005–2006). Only 5% of the households in India were covered under any kind of health insurance. Within the insurance schemes, the state owned health schemes are the most subscribed (39.2), followed by the Employee State Insurance Scheme (17%). Among the households belonging to the lowest economic categories, <3% were covered by any health scheme or health insurance. However, the recent trends show that the community health insurance targeting poor households are becoming much popular and it may be the most appropriate way of supporting the families vulnerable to catastrophic health spending.

## 4.3 Research capacity development

## 4.3.3 Global Health Research and Education: what is it, and how should it be taught?

### O.4.3.3.001
**Global Health Selective: a novel interdisciplinary clerkship on clinical knowledge and skills for global health at New York University School of Medicine**

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INTRODUCTION Global health (GH) spans every scientific, clinical and social science discipline. Cultural competency/cross-cultural sensitivity has been identified as a GH priority for U.S. medical schools (Peluso, 2013). As part of Curriculum for the 21st Century (C21), the Global Health Selective is part of the new Global Health Concentration at NYU School of Medicine (SoM). With special emphasis on cultural competency/cross-cultural sensitivity, its primary aim is to teach future physicians fund of knowledge and clinical skills that strengthen GH care.
METHODS As a 4-week clinical clerkship, the GH Selective was first completed by nine medical students in 2012, and again by 12 medical students in 2013. Activities included 18 90-min patient case discussions in tropical medicine; related clinical assignments at NYU; literature review and journal club; and nine half-day clinical skills simulation workshops covering 1) diarrhea in Haiti and Egypt, 2) tuberculosis in Peru, 3) malaria in sub-Saharan Africa, 4) hypertension screening by community health workers in Ghana, 5) survivors of torture from central Africa, 6) humanitarian response to a tsunami in Indonesia, 7) obstetrical emergencies in rural Liberia, 8) interpreter exercise in Tibetan, and 9) smoking cessation via interpreter. Leadership is from NYU SoM Departments of Medicine and Population Health, and Center for Healthful Behavior Change.
RESULTS Over both years of the GH Selective, student feedback was overwhelmingly positive. Each year, at least 37 faculty volunteered from 11 departments at SoM to log at least 225 h of direct contact teaching hours.
DISCUSSION The GH Selective exceeded expectations. Its interdisciplinary curriculum is a particular strength, and its special emphasis on working with standardized patients in cross-cultural settings, with special focus on communication skills, health literacy, and health navigation, provided students with knowledge and clinical skills applicable for any clinical care provided locally, nationally, and worldwide.

## 4.5 Health systems in conflict and disaster areas

### O.4.5.1.001
**Haïti and the health marketplace: the results are perishable**

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INTRODUCTION In most societies the health marketplace is pluralistic in character, with a mix of formal and informal providers. In advanced economies, state regulation of the market helps ensure quality and access and helps mitigate market failures. In the present study we explore what happens to the functioning of the pluralistic health marketplace when the state is weak and the informal sector is able to flourish, using Haïti as a case study.
METHODS The research methods included an extensive documentary and policy analysis, based on peer-reviewed articles, books and 'grey' literature—government policy and program reports, unpublished research and evaluations, reviews and reviews from key multilateral and bilateral donors, and non-government organisations, combined with field site visits and key informant interviews ($N = 45$).
RESULTS The findings show that despite considerable investment, donor policies have largely failed the Haïtian population in terms of meeting their basic health needs as reflected in the deplorable health indices. What gains have been achieved are rapidly eroded: 'les resultats sont périssables' (the results are perishable).
CONCLUSIONS Working in fragile states with limited capacity to undertake the core function of securing the health of its population requires new and innovative ways of working. This needs longer timeframes, combining incremental top-down and bottom-up strategies which recognize and work with state and civil society, public and private actors, formal and informal institutions, and progressively facilitate changes in the different market functions of supply and demand, and regulation and supporting functions.

### O.4.5.1.002
**Prevalence and risk factors for adult malnutrition in a post-conflict area, Northern Uganda**

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INTRODUCTION Health data is scares in the post-conflict vacuum of northern Uganda. The objective was to determine the prevalence of adult malnutrition and associated risk factors including exposure to conflict, displacement and World Food Programme (WFP).
METHODS A population-based cross-sectional study was performed from September 2011 to March 2013. All residents in Gulu Health and Demographic Surveillance System aged >15 years were eligible. Trained assistants measured weight and height, and administered questionnaires with socio-demographic information, food security, smoking, alcohol, food rations from

WFP, and camp displacement. Nutritional status was classified by body mass index and short stature.
RESULTS One thousand eight hundred and seventy-four men and 2649 women participated in the study (participation rate of 66.6%). for men and women respectively, the prevalence of malnutrition was: underweight 23.8% and 17.7%, overweight 1.4% and 7.0%, and short stature 8.3% and 1.8%. In men, underweight was associated with younger and older age ($P < 0.001$), being divorced/separated [OR 2.12 (95%CI 1.33;3.42)], single [OR 1.61 (95%CI 1.01;2.60)] and daily smoking [OR 2.04 (95%CI 1.58;2.65)]. for women, underweight was associated with older age ($P < 0.001$), rainy season [OR 1.26 (95%CI 1.02;1.55)], and having unstable main source of food ($P = 0.003$). Displaced more than once was associated with a protective effect for underweight ($P = 0.026$ for men and $P = 0.05$ for women). Being born during conflict was associated with having short stature in both men and women (OR 25.77 (95%CI 12.40;53:63) and OR 6.05 (95%CI 1.66;22.11).Exposure to WFP and years displaced was unrelated to underweight.
CONCLUSIONS Malnutrition was common as underweight, but varied between gender and ages -young men and elderly were at particular risk. This finding was not in line with the present focus in humanitarian assistance on children and women. Nutritional assessments prior to humanitarian assistance, and continuous monitoring of all population groups could help mitigate such disparities and improve health in post-conflict areas.

### O.4.5.1.003

**Access to effective primary care and referral for patients in Afghanistan problematic due to health service performance and specific financial, geographic and insecurity barriers**

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INTRODUCTION Donor support to the Afghan health system privileged contracting out of services to national and international agencies. Certain agencies envisage introducing user fees for essential care on the short term. Support to the health sector is seen by some international actors as a transformative tool for state building and stabilization, which raises concerns. In particular, perception of NGOs as partial to the political or military arena could increase security risks. Recently the validity of formal reports on the coverage and performance of the Afghan health system have been put into question. Ideally population based surveys would be indicated as alternative source of information. However due to insecurity, an alternative approach was used.
MATERIAL AND METHODS In four locations in Afganistan patients ($n = 120$ per site) were interviewed on basis of a standard questionnaire were held with patients visiting MSF supported health structures or specific services within hospitals. The questions focused on access obstacles during the current referral period and enquired also about health seeking behaviour during previous illness episodes in the household.
RESULTS AND CONCLUSIONS Preliminary data as analysis not completed. In spite of the bias linked to the methodology towards people accessing the services and potentially underestimating access problems, the interviews revealed patients face important access obstacles. Besides geographic barriers, financial access and functionality of effective care by health structures in the periphery proved problematic. These results indicated important divergence from the formal reporting system in the area. Households remain in very precarious socio-economic situation; introducing patient fees carries an important risk of further deterioration of affordable and available health services.

## 4.10 Sanitation

## Sanitation in the post 2015 landscape

### O.4.10.001

**Waste handling practices and common health problems among occupational waste handlers in Prampram, Ghana**

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INTRODUCTION Inappropriate waste (liquid, solid and human faecal waste) handling may pose health problems to those occupationally involved. In peri-urban communities in Ghana, the acute problem with open defecation and inadequate waste removal results in widespread human excreta and solid waste pile up. There is, however, little scientific evidence on health problems of waste handlers. This study investigated waste handling practices and perceived health problems among waste handlers in Prampram, a coastal community in Ghana.
METHODS Researcher-administered questionnaire was used to obtain information about waste handling practices and associated self-perceived health problems from all 280 occupational waste handlers in Prampram, Ghana. In-depth interviews were conducted for 22 of these waste handlers.
RESULTS The major waste handling practices were sweeping (74.6%; 209/280), collection (72.1%; 202/280) and disposal (50.4%; 141/280) of waste. Some occupational waste handlers performed multiple waste handling tasks. The majority (77.9%; 218/280) of these waste handlers who spent 4–6 h/day on waste handling activities were collectors who also more frequently used personal protective gears (PPGs); i.e. 84.3% (172/204) used overall gown, 83.4% (146/175) used Wellington boots, 76.5% (127/166) used gloves and 76.7% (69/90) used oro-nasal masks. The most common self-reported disease symptoms were fever 35.7% (100/280), headache 38.6% (108/280), body pains 56.4% (158/280), diarrhoea 11.4% (32/280) and 'itchy anus' 22.7% (5/22). Occupational waste handlers who reported fever (77.0%; 77/100), headache (77.8%; 84/108) and diarrhoea (81.3%; 26/32) were predominantly sweepers whilst most collectors (81.0%; 128/158) reported body pains. Working for 4–6 h/day seems to increase self-reported body pains among waste collectors compared with sweepers (72.2%; 114/258) and disposers (52.5%; 83/158).
CONCLUSION Occupational waste handlers perceive their occupation as resulting in common work-related health problems. There is need to determine actual health risks among waste handlers and implement health promotion measures to reduce health risks associated with waste handling.

### O.4.10.002

**Sanitation business in peri-urban communities – the case of southern Ghana**

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In spite of efforts made, Ghana still lags behind the MDGs' target for sanitation. The country's current sanitation policy


 

supports the private-led approach to promoting improved sanitation. To understand the operations of the private sector in the sanitation market, this study sought to identify and describe sanitation businesses, particularly toilet-related businesses, in poor peri-urban communities in southern Ghana. Participants for the study, including small toilet-related businesses, public toilet managers, local authorities/district officers and households, were selected using purposive and snowball sampling techniques. Data were generated by observations, Focus Group Discussions, and key-informant and in-depth interviews. Data were analysed by transcribing and interpreting audio recordings, as well as the use of content analysis. The importance of business constraints was assessed using Kendall's concordance analysis. Results of the study showed that there are various kinds of sanitation-related businesses/operators in the study area, including solid waste collection, toilet management, hardware suppliers, local masons/toilet builders, pit emptiers, and commercial bathhouse operators and water sellers. Most of the businesses were not registered, and they use the service contract business model, where operators are hired to perform specific services for a fee. In addition, they also employ business models like the partial private-public partnership, micro-franchise/rehabilitate-and-operate, networking and one-stop-shop. The effectiveness of business performance as perceived by the respondents was estimated as below average. However, the businesses obtain a profit margin of 10–30% on the services they provide. Among the constraints influencing business performance include difficulty in accessing credit, irregular cash flows, delayed payment for services, and institutional impediments. To improve sanitation and ensure sustainability of the businesses, there is the need for better cooperation between operators and their allied stakeholders. There is also the need for public education on the importance of proper sanitation.

# Track 5: Global Interaction and Health

## 5.2 Global interaction and health

## Migration and neglected parasitic diseases

### O.5.2.001

**Performance of rapid serological diagnostic tests for infection with *Trypanosoma cruzi* with serum/plasma/whole blood in Chagas disease endemic and non-endemic countries**

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Chagas disease is one of the main Latin American public health problems. In last decades, mainly due to population movements, *T. cruzi* infection has increasingly been detected in North America, Europe and Western Pacific. One of its biggest control challenges is its underdiagnosis index in the world (<10%). Lack of evaluation of rapid serological diagnostic tests is one of the limitations to expand detection and increase treatment coverage either in endemic and non-endemic areas. The study aimed to evaluate the performance of commercialized rapid serological diagnostic tests (RDT) for *T. cruzi* infection with serum/plasma and whole blood, initially in collaboration with 11 national reference laboratories (NRL) of different world areas. Based on the performance results of the initial phase, four of the best tests were evaluated in Aiquile, Bolivia, focusing on its performance on whole blood under field condition. In the initial phase, 11 commercialized RDTs for *T. cruzi* infection were tested with 500 samples from the serum banks of NRLs, previously tested for Chagas disease. The sensitivity, specificity and concordance of each RDT were measured. Simultaneously, potential cross reactions (i.e: Leishmaniasis, malaria, HIV) were evaluated. Test results were classified in the following three categories: positive, negative, and invalid/indeterminate, following strictly the definition provided by manufacturers. The 11 commercialized RDTs were consequently classified in three categories: high, medium and low performance. Among the evaluated RDTs for Chagas disease, the majority of them were classified in the medium performance category, 25% were classify as low performance and 20% as high performance. In the Bolivian field assessment, results of the four selected RDTs showed sensitivity and specificity values above 95%. In line with the promotion of access to diagnosis and treatment of chronic Chagas disease, the results showed that RDTs can be useful an alternative and reliable instrument.

### O.5.2.002

**If you don't take a temperature you can't find a fever – the problem of unrecognized infections in children migrating to Europe from low income countries (LICs)**

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INTRODUCTION The published epidemiology of infectious diseases (IDs) and data on the interaction between health services and migrating children from LICs speak in favour of considerable unmet health needs. The IDs diagnosed after arrival pose a risk mainly to the migrant children and their communities and not or to a minor degree to the population of the host country.

MATERIALS AND METHODS Literature search and assessment of published data.

RESULTS Migration from LICs to Europe has increased in the process of globalization. In migrants, including children, WHO data documents a higher prevalence of virtually all IDs in LICs compared to high income countries (HICs). Since the spectrum of IDs in migrants depends on their region of origin, there is no 'one size fits all' approach. Access of migrant children to the health system of the receiving HICs may be restricted; and even those entitled to health services consult them less than their native counterparts. Those consulting the health system often find providers lacking specific training or information resources to adequately address their specific needs. A literature search for terms representing childhood, migration and infectious diseases locate the bulk of research on the topic in „classical' immigration countries such as the USA, Canada and Australia. Here standardized diagnostic approaches are also being tested. European countries constitute important destinations for worldwide migration. Measured by the number and type of publications little attention is being

paid to the improvement of health of immigrant children from LICs.
CONCLUSION The access to health care of children migrating from LICs to Europe and the ability of health care providers to meet their specific needs are unsatisfactory.

## 5.4 Infectious disease and public health in Greenland

### O.5.4.001
**Hepatitis B infection in Greenland: epidemiology and burden of disease**

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BACKGROUND Greenland remains a high-endemic country for hepatitis B (HBV) infection with 5–10% of the population being HBsAg positive (chronic carriers). Surprisingly, despite of the high prevalence of HBV infection, acute and chronic hepatitis B, liver cirrhosis and primary hepatocellular carcinoma appear much less frequently than expected. The reasons for the low frequencies are unknown, but as a consequence implementation of a childhood HBV vaccination programme, though debated for years, was not included in the childhood vaccination programme in Greenland until September 2010. In this presentation we describe the epidemiology and the clinical burden of HBV in Greenland.
MATERIAL AND METHODS 1) In total, 8879 Greenlanders (16% of the total population) from population-based surveys in 1987 and 1998 were followed through March 2009. Information on morbidity and mortality by HBV status was obtained by linkage to the Patient Discharge Registry, the Cancer Registry and the Central Registration System. 2) All inhabitants in a HBV-hyper-endemic settlement with an unexpectedly high number of acute hepatitis cases were invited for follow-up from 2005 to 2009 to elucidate the reason for acute hepatitis cases.
RESULTS We found an increased incidence rate ratio in first time hospitalisations of non-alcoholic cirrhosis and HCC for HBV-chronic carriers versus HBV-negative persons, but the age-standardised incidence rates for the diseases among chronic carriers in Greenland were low compared with other populations of chronically HBV infected persons. However, in the settlement an ongoing hepatitis D (HDV) outbreak among chronically HBV-infected children seems to cause acute hepatitis.
CONCLUSIONS The low incidence of HBV-related diseases among chronic carriers suggests a more benign course of HBV in Greenland than in other parts of the world. However, the HBV-HDV super-infection in the settlement challenges the notion that HBV infection may not be as harmless in certain areas as previously anticipated.

## 5.5 Occupational and environmental health in a globalized world

### O.5.5.001
**Evaluation of an integrated pest management intervention among Ugandan small-scale farmers**

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INTRODUCTION In developing countries, the use of hazardous pesticides in agriculture has increased. Often farmers are not trained in how to use and handle pesticides, which leads to unnecessary occupational exposure. Methods of integrated pest management (IPM) have shown to reduce the use of hazardous pesticides without affecting the yield. In order to reduce pesticide use and exposure a large-scale training project was started in 2010 in Uganda. The aim of this study was to evaluate the effect of an IPM intervention among Ugandan small-scale farmers.
METHOD AND MATERIALS In 2010, a group of 40 farmers from two project areas were chosen by their farmers' group to take part in a 2 year IPM intervention. The trained farmers were instructed and supervised in teaching their fellow farmers. By the end of the intervention in November 2012, 35 trained farmers, 46 farmers trained by the trained farmers and 33 control farmers went through a standardized interview on pesticide related acute symptoms and knowledge, use, practice, and attitude. The cross sectional data were analysed using chi-square- and trend test between the three groups.
RESULTS AND CONCLUSIONS The analyses showed that the trained farmers, compared to the fellow farmers and the control farmers, knew more about ways of pesticide exposure, environmental side effects, alternatives to pesticides and colour codes for hazardousness. They used more protective gear, had a better pesticide hygiene and their attitude towards pesticides reduction was more positive. A trend between groups was found on many parameters indicating that trained farmers passed on their knowledge to fellow farmers. There was no significant difference on spraying frequency, type of pesticide use, or acute symptoms, which may indicate that more training is needed.

### O.5.5.002
**Large scale mercury pollution from small scale gold mining – a problem with a solution**

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INTRODUCTION Mercury is used globally to extract gold by artisanal small scale gold miners (ASGMs). 'Whole-ore-amalgamation' is an unfavorable technique where 15–40 g of mercury is consumed to produce one gram of gold. One third of the global human mercury emission may derive from ASGM in the Philippines and Indonesia where this method is practiced. However, ASGMs from the Benguet region in the Philippines have been using the non-toxic mercury-free borax-method for gold extraction the last 15 years. We present the preliminary results from a 3 years project with the aim of introducing the borax-method in mining areas using whole-ore-amalgamation.


 

MATERIAL AND METHODS We intervened with a civil society strategy on two mining communities in Kalinga and Camarines Norte with approximately 2000 ASGMs associated. The intervention was based on miner-to-miner training, training of health- care staff, school teachers, and school children. Throughout the project we worked hard to involve the local political leaders. A baseline survey was conducted in 2011 among ASGMs, health-care professionals, and representatives from civil society. The follow-up study is planned for December 2013.
RESULTS In October 2012, 70% of the ASGMs in Kalinga had converted to the borax-method, whereas only a handful had changed method in Camarines Norte. Monitoring of the gold extraction processes has shown that the borax-method yields the same amount or more gold than the mercury method, but it is more labor intensive. In Kalinga we managed to involve the politically influential tribal organization, whereas in Camarines Norte we did not manage to find a suitable structure in the society to work with.
CONCLUSIONS The borax-method is doable and the civil society strategy for implementing it is possible. However involvement of the local leaders in the municipality or another strong local organization appears to be crucial.

## O.5.5.003

### Mercury exposure in artisanal small-scale gold mining: symptoms, knowledge and incentives for change

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INTRODUCTION Approximately 10–15 million artisanal small scale gold miners (ASGMs) world-wide are exposed to vapours from mercury. Metallic mercury is a neurotoxin inducing severe acute and long term effects. In relation to an on-going intervention project in the Philippines where ASGMs are trained to use a mercury-free gold extraction method we reviewed the literature on mercury exposure and conducted a study on symptoms, knowledge and practise.
MATERIAL AND METHODS We searched relevant databases for existing studies regarding mercury and mining. We extracted relevant data on urine concentrations in miners working in mining, milling, smelting, and refining and for residents and related the information to existing guidelines for thresholds in the working environment. e conducted structured baseline interviews of miners in an intervention area ($n$ = 208) and in a control mining area ($n$ = 50) where the mercury-free method had been in use for 15 years. The interviews focused on mercury-related symptoms, knowledge and practise and priorities for change of method.
RESULTS AND CONCLUSION In our literature search we found 26 studies covering 14 countries. In all, three thousand and five residents and miners and 442 controls had been examined. Workers, especially smelters and refiners, had consistently higher urine levels than residents who had higher levels than controls. 12.6–81.7% had levels exceeding existing guidelines. Our baseline study showed a trend towards increasing levels of mercury-related symptoms among exposed miners compared to miners with no direct exposure and controls. 94.8% of the exposed miners took precautions but these were inefficient. They prioritized health if they were to change method but not pollution. In conclusion, ASGMs are exposed to mercury levels that may cause acute and irreversible effects. Possibilities of precautions are few and inefficient and the knowledge among miners regarding mercury toxicity and exposure pathways are low and will be addressed in the intervention project.

## O.5.5.004

### Prevalence of Hepatitis B and C among health care professionals in a tertiary hospital in Tanzania

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BRIEF INTRODUCTION Health care professionals have a high risk of contracting Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infection from their work place especially in resource limited settings. This study aimed to investigate the prevalence and the risk factors of HBV- and HCV-infection in employees of a tertiary hospital in northern Tanzania.
MATERIAL AND METHODS The study enrolled 600 health care workers (HCW) aged ¡Ý16 years at the Bugando Medical Centre, Mwanza, Tanzania. HBs antigen, anti-HBc, anti-HBs and HCV antibodies were determined from 598 evaluable serum samples. HCV antibody positive samples underwent PCR for HCV-RNA. The HCW were divided into risk health care workers (rHCW) highly exposed to infectious materials like needles and non-risk-health-care-workers (nrHCW). Potential risk factors were recorded in a standardized questionnaire.
RESULTS AND CONCLUSION Four age groups were defined (Table). Three hundred and sixty participants (60.2%) were female. Four hundred and seventy-three (79.2%) employees belonged to the rHCW, 125 (20.8%) to the nrHCW. The overall prevalence of HBs antigen and anti-HBc antibodies was 7.2% (43/598) and 48.7% (291/598), respectively. There was a significantly higher risk of contact with HBV identified by estimation of anti-HBc odds ratios in different age groups reflecting the years of working experience. The results for anti-HBc odds ratio of age 51–65 years compared to age 16–30 years were in the rHCW 3.297 ($P < 0.0001$) versus 1.385 ($P = 0.606$) in nrHCW. Among the HCW there is a significant increase of anti-HBc positivity in rHCW (49.6%) versus nrHCW (34.2%; $P = 0.046$ Fisher's Exact Test). HCV antibody prevalence was 1.2% and HCV-RNA 0.3% without significant difference in rHCW and nHCW ($P$-value HCV-AB: 0.668, HCV-RNA: 0.309). There was no HBV-HCV. co-infection. This study demonstrates a significantly increased risk of Hepatitis B infection for HCW due to professional exposure. HCW in Tanzania should be offered free HBV vaccination therefore.

### O.5.5.005

**Occupational health and safety in Ghana – illustrated by a case study in a rubber plantation**

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INTRODUCTION The agricultural sector is one of the most hazardous sectors in Ghana and other developing countries. Despite of the existing occupational health and safety (OHS) legislation in Ghana this sector is still not well covered and no systematic workplace assessments (WPA) are carried out. The purpose of the study was to assess the working environment at the largest rubber plantation in Ghana using a participatory method and to evaluate the applicability of this method in a workplace characterised by unskilled workers.
MATERIALS AND METHODS The used method for WPA included observations, individual interviews and focus group discussions (FGD). Five field workers and 1 headman from four different work functions (spraying, slashing, budding and tapping) and three managers were interviewed individually. The individual interviews were based on the WPA check lists from Denmark and the international WPA programs WIND and WISE. The same field workers participated in the FGD to identify the most important hazards in the plantation and possible solutions.
RESULTS The present WPA identified several general occupational hazards including deep holes hidden by weed in the plantation, excessive heat, lacking supply of clean drinking water and lack of first aid equipment. Also, specific hazards within each work function were identified, e.g. repetitive and forceful work in weeding. Based on these results recommendations for the rubber plantation were made.
CONCLUSION The present study supports the idea of involving the employees in the working environment procedures. The method was an efficient way to address OHS in a workplace characterized by unskilled workers and serious hazards, but for future WPA's in developing countries the emphasis should be put on observations and FGD as the individual interviews were time consuming and is suggested replaced by more systematic observations.

### O.5.5.006

**Laboratory exposure to *Brucella melitensis* in Denmark: a prospective study**

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INTRODUCTION Brucellosis is one of the most common laboratory acquired infections as Brucella species are easily transmitted by aerosol-generating procedures. This is especially seen in laboratories serving non-endemic areas and therefore unfamiliar with the organism. We describe the handling of a laboratory exposure to Brucella melitensis in a large microbiology laboratory in a Brucella non-endemic area.
METHODS A total of 17 laboratory staff members were exposed to B.melitensis. Exposure was handled according to the US Centers for Disease Control and prevention (CDC) guidelines. Serology testing was performed by a Brucella agglutination test at baseline, week 6 and week 24 after exposure.
RESULTS A total of 14 of the staff members were classified as high-risk exposure and 3 as low-risk exposure. None of the staff members accepted post exposure prophylaxis comprising of doxycycline and rifampicin. Staff compliance for the recommended serological follow-up protocol was 88%. In a period of 6 months follow-up none of the exposed laboratory workers developed brucellosis and all obtained sera were negative for anti-brucella antibodies. However, in the first 8 weeks after exposure to Brucella, 4 of the 17 staff members were investigated due to fever and suspicion of brucellosis.
CONCLUSIONS In the management of laboratory exposure to Brucella surveillance of clinical suspicion of brucellosis in exposed laboratory personnel seems of importance in order to their timely diagnosis and treatment if symptoms occur. The value of routinely serological follow-up seems less obvious.

# Track 6: 5th Conference of the Scandinavian-Baltic Society for Parasitology

## 6.2 Parasites as a cure for human diseases/ disorders

## Worms as a therapy for human immune mediated diseases

### O.6.2.001

**A monitored clinical course of *Trichuris trichiura* self-infestation complicated by infectious *Campylobacter gastroenteritis***

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INTRODUCTION Helminth therapy is increasingly used as immune modulators in auto-immune related diseases. In order to distinguish host immune reactions secondary to the helminth infestation from disease-related mucosal responses with parallel clinical improvement, we monitored a clinical course of helminth infestation in a volunteer without prior intestinal disease.
MATERIAL AND METHODS A healthy male volunteer underwent colonoscopy and delivered venous blood and faecal samples before, during, and after ingestion of a total of 1250 *Trichuris trichiura* eggs. Colonisation was documented by eosinophil counts, faecal microscopy and inspection during colonoscopy. Colonic mucosal mRNA for T cell related cytokines was analysed by qPCR.
RESULTS Fifteen weeks after first *T. trichiura* ingestion egg count was 6260 eggs per gram of faeces was paralleled by a rise in circulating eosinophils to a maximum of 2.26/nl. Faecal calprotectin remained under the detection limit throughout the infestation. At colonoscopy the presence of *T. trichirua* was limited to the ascending and transversing colon. Following parasite eradication using a 5-day mebendazole treatment, the volunteer developed abdominal pain and erythema at follow-up colonoscopy 8 weeks later. Faecal cultures grew *Campylobacter jejuni*. Concomittantly, faecal calprotectin rose to 830 mg/kg faeces. Campylobacter infection disappeared spontaneously after the colonoscopy. Mucosal expression of mRNA for T cell


 

related cytokines in colonic biopsies taken during *T. trichiura* infestation was markedly up regulated (up to 105-fold higher) compared to the expression at baseline and during Campylobacter infection. The up regulated expression was only seen in the colonic regions in which *T.* trichiura was present, and thus displayed marked regional variation.
CONCLUSIONS Circulating eosinophils, as opposed to faecal calprotectin, reflect the degree of colonisation. Colonic infestation results in regional activation of mucosal immune response reflected mucosal mRNA for T cell related cytokines. The infested host may be susceptible to accidental Campylobacter superinfection.

### O.6.2.002

#### *In vivo* hatching and initial establishment of *Trichuris suis* eggs in Göttingen minipigs

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*Trichuris suis* (*T. suis*), the pig whipworm, colonizes the cecum and proximal colon. The eggs of *T. suis* eggs (TSO) are tested by others as the active pharmaceutical ingredient against inflammatory bowel disease in human patients. *In vivo* infection in Göttingen minipigs is a presently available model for assessment of biological potency of *T. suis* eggs. This study aims at exploring an early phase of *T. suis* infection under controlled conditions in Göttingen minipigs infected with high infection dose at 2, 4, 6, 8, 12 and 24 h after infection. Hatching of whipworm eggs and the early establishment of the first stage larvae in the intestinal mucosa will further provide insight into the location and the role of intestinal environment in hatching processes. Thus, identification of main factors involved in hatching processes is important in expanding our knowledge on host-parasite interaction and for development of complementary *in vitro* systems.

## 6.3 Parasitic zoonoses/diseases in the Scandinavian-Baltic region

### 6.3.1 *Echinococcus multilocularis*

### O.6.3.1.001

#### Three case reports of liver alveolar hydatidosis and an overview of this infection in the Czech Republic

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INTRODUCTION The first case of liver alveolar hydatidosis (AH) was described in the Czech Republic (CZ) post mortem at 74 years old patient in 1979. There were not diagnosed more human cases of this disease until recently, although infection of foxes with Echinococcus multilocularis is common in our country. During 1994 and 1998, there were sacrificed more than 1500 foxes from entire CZ and the prevalence of this parasite was 2.5–12.5% of depending on region. Since 2007 there were diagnosed at least 10 cases of liver AH in CZ. Here, we are presenting three case reports of the liver AH.
CASE REPORTS A 30-year old woman detected a palpable mass in the right hypochondrium in December 2011. The CT-scan and MRI revealed enlargement of the liver and cystic mass ($120 \times 90 \times 60$ mm) in both right and left liver lobes. The typical morphology and positive serology confirmed the diagnosis of AH. The liver lesion was inoperable, therefore patient is on continuous therapy with albendazole. The second case is a 30-year old woman who detected resistance in her right hypochondrium in 2009. The US and CT-scan revealed a liver mass which was described as haemangioma. The patient suffered with sclerosis multiplex, biological therapy was iniciated and the mass enlarged. The CT-scan and MRI revealed three multilocular lesions in June 2012 and AH was serologically confirmed. This patient underwent a palliative surgery and she is on continuous albendazole treatment. The third patient is 60-year old lady who was sent to our hospital with AH diagnosed after laparotomy, liver biopsy and typical parasite histology. Serology was positive for AH. The lesion is inoperable and patient is on albendazole therapy.
CONCLUSIONS AH is endemic in CZ and there are reported increasing numbers of cases. Diagnosis of AH is difficult in some cases.

### O.6.3.1.002

#### Chemokine responses in patients with cured, stable and progressive Alveolar Echinococcosis

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INTRODUCTION Alveolar Echinococcosis (AE) is caused by the tapeworm *Echinococcus multilocularis* in humans, which may lead to massive larval (metacestode) growth and ultimately to organ failure. In some AE patients, however, metacestode proliferation can regress. Several studies focussed on the host-parasite interplay and immune modulation as induced by the parasite to evade the host's immune response thus facilitating the survival and proliferation of the metacestode.
METHODS Peripheral blood mononuclear cells (PBMC) and plasma were collected from AE patients (cured, stable, progressive) and infection-free controls. The cellular chemokine production of Eotaxin 1, 2 and 3 as well as SDF-1/CXCL12 was studied by ELISA aimed to identify immune response profiles in AE patient groups. Furthermore, chemokine concentrations in plasma samples of AE patients and controls were investigated.
RESULTS In all AE patient groups, *E. multilocularis* antigens induced elevated levels of Eotoaxin-2/CCL24 by PBMCs, and similarly, the spontaneous release of pro-inflammatory Eotaxin/CCL11 and Eotaxin-2/CCL24, and Eotaxin-3/CCL26 were higher in AE patients than controls. Also the *E. multilocularis* vesicle extract enhanced the cellular production of CCL11, CCL26 and SDF-1/CXCL12. In AE patients, Eotaxin-2/CCL24 responses were also activated by Ascaris lumbricoides antigen (AscAg), but AscAg inhibited production of CXCL12. In patients with progressive AE the highest levels of CCL11, CCL26 and CXCL12 were observed, but plasma levels of CCL24 were lower in AE than in controls.

CONCLUSION This study demonstrated distinct chemokine response profiles at different stages of AE, and this may provide a better understanding of the host-parasite interplay and for better staging of AE patients.

## O.6.3.1.003

### Changing paradigms for *Echinococcus multilocularis* in northern North America

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*Echinococcus* spp. cestodes are emerging and re-emerging as One Health concerns across the circumpolar North. In northern North America, *E. multilocularis* is established in the northern tundra zone, where the sylvatic definitive hosts were thought to be Arctic fox, and the central North American prairies, where the most important sylvatic definitive hosts were thought to be red fox. Our recent work suggests that wolves are an equally or perhaps more important definitive host than Arctic fox in the mainland North American Arctic. Similarly, in central North America, coyotes are proving to be a more important definitive host than red fox, especially in periurban areas where the parasite is spilling over into domestic dogs. We report three sentinel cases of alveolar hydatids in dogs, which are generally thought to serve as definitive, not aberrant intermediate, hosts. In 2 of the clinically affected dogs, European-type strains of *E. multilocularis* were detected, compatible with reports of dogs serving as aberrant intermediate hosts in highly endemic regions of central Europe. We recently reported the establishment of a European-type strain of *E. multilocularis* in a forested region on the west coast of Canada, 600 km west of the nearest previous report, suggesting a possible recent introduction with imported dogs or red fox. Finally, in rodent intermediate hosts in the Central North American region, characterization of alveolar hydatid cysts at multiple mitochondrial loci displayed unexpectedly high genetic diversity. This suggests that this region represents a highly endemic, well-established focus of *E. multilocularis* and not, as previously suggested, a recent introduction from the Arctic. These studies challenge established paradigms about this important parasite in North America, and identify host and range expansion of endemic and introduced strains of *E. multilocularis*, possibly as a result of increased globalization and environmental change affecting the entire circumpolar North.

## O.6.3.1.004

### Updates on the surveillance program on parasites of raccoon dogs and foxes in Denmark 2011–2012

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Raccoon dogs have recently invaded Denmark, which marked concern about potential introduction of parasites to native species. In the same time, a nation-wide surveillance program was initiated to screen red foxes for presence of *Echinococcus multilocularis*. Here, we present the results of that surveillance study, which included analyses of gastrointestinal helminths and *Trichinella* spp. in 99 raccoon dogs and 384 foxes collected from October 2009 to March 2012. Raccoon dogs and red foxes harbored nine and 13 helminth species, respectively, many of which are potentially zoonotic. While all animals examined were Trichinella-free, a fox harbored 20 worms of *E. multilocularis* (0.3%). Parasites of raccoon dogs were mainly rodent-transmitted, while parasites of red foxes were mainly amphibian-transmitted, which may suggest less important role of raccoon dogs in the transmission of *E. multilocularis*. Differences in the prevalence, abundance and intestinal distribution of several parasite species were evident between the two host species. Flukes of Alaria alata in raccoon dogs were very more prevalent and smaller in size than those recovered from foxes. In raccoon dogs, results of multivariate analysis showed that the abundances of *Mesocestoides* spp., *A. alata* and *Cryptocotyle* spp. were season-associated, while the abundance of *Cryptocotyle* spp. was also associated with the age of hosts. In foxes, regression parameters revealed increased incidence of *Uncinaria stenocephala*, *A. alata* and *Pygidiopsis summa* in adult foxes, increased incidence of *Toxocara canis*, *A. alata* and *Mesorchis denticulatus* in Jutland compared to other islands in Denmark, and increased incidence of *T. canis* and *Cryptocotyle* spp. in male foxes. Many biological factors may have shaped the observed differences between helminths of raccoon dogs and foxes. The results of this study showed the importance of surveillance programs in early discovery and monitoring of zoonotic infections in native and invading animals.

## O.6.3.1.005

### Present status of *Echinococcus multilocularis* in Sweden

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Surveillance of *Echinococcus multilocularis* (Em) has been done in Sweden since 2000. Until 2010, all samples (2962 red foxes, 68 raccoon dogs and 35 wolves) had yielded negative results. In 2011, the first positive fox was found, shot in Västra Götaland county. To clarify prevalence as well as the distribution of the parasite an extended surveillance of foxes was initiated. By the end of June 2011, a total of 2985 hunted foxes had been collected and analysed with the segmental sedimentation and counting technique (SSCT). Three positive foxes were found, one in the previously known infected area, one in Södermanland county and one in Dalarna county. Based on this study, it was concluded that the prevalence of the parasite was very low, approximately 0.1% but that Em foci were spread over a large part of the country. Em was considered to be endemic in Sweden and not possible to eradicate. It was considered most probable that the parasite was introduced in Sweden by infected dogs due to relaxation in import requirements. An intensive surveillance in the infected area of Södermanland was initiated in 2011. In all, 790 fox scats were collected within 2000 hectares and analysed with a MC-PCR. Six samples (0.8%) were found to be infected. In 2012–2013, a second country wide surveillance was initiated, using collected fox scat samples. Study results will be used as a baseline for comparison with future studies, to evaluate any change in prevalence and spread. The risk for humans to become

infected in Sweden is considered to be low and consumers are informed that good hygienic practice when handling food also applies to Em. Alveolar Echinococcosis had never been reported in Sweden until 2012, when the first two human cases were diagnosed. However, both cases were considered as having been infected abroad.

### O.6.3.1.006

**Ghost-hunting – is *Echinococcus multilocularis* really absent from mainland Norway?**

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The Norwegian Veterinary Institute has carried out active surveillance for *Echinococcus multilocularis* (EM) in red foxes since 2007 and passive surveillance since 2002. *Echinococcus multilocularis* has not been detected in any of the 2782 fox faecal samples examined in the period 2002–2012. In 2012, we carried out studies to compare molecular copro-methods for EM detection. The egg isolation technique, with duplicate manual sieving stages, and manual column DNA extraction followed by a multiplex PCR, was compared with a new DNA fishing method followed by detection using one of three different real-time PCR methods. The improved sensitivity of the new methods (approximately 60% sensitivity with the new methods compared to 30% with the old) together with additional benefits such as closed tube detection and detectability of proglottid/adult stages in the material, resulted in the new system being adapted for use in the surveillance program as of 2012. The parasite was not detected in the 616 samples examined using this new method (DNA fishing followed by detection through realtime PCR). Despite the poorer sensitivity of the old m-PCR method, than originally estimated, the methods used (both old and new) indicate that the prevalence level in red foxes in Norway, for the previous 5 years, is below 1%. This detection level is given by EFSA as the critical level for eligibility for the maintenance of national regulations for pre-import tapeworm treatment of dogs. Accordingly, Norway is therefore still able to demand compulsory prophylactic tapeworm treatment of all dogs entering the country (except from listed EM free countries). However, given the extremely low prevalence of EM reported in foxes in neighbouring Sweden, <0.001%, EM may still be present in Norway, but at levels far below the current detection level of our surveillance program, currently set at 1%.

## 6.3.2 Fish-borne parasitic zoonoses in Europe

### O.6.3.2.001

**Emerging *Pseudoterranova decipiens* problems in Baltic cod (*Gadus morhua* L.) associated with grey seal colonization of spawning grounds**

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Zoonotic nematode larvae of the species *Pseudoterranova decipiens* (cod worms, seal worms) are prevalent anisakid nematode larvae in many fish stocks in the Atlantic and the Pacific but have until recently been absent or extremely rare in the isolated and stationary Baltic cod population located in the Baltic Sea between Denmark Germany, Poland, Sweden, Finland, Russia and the Baltic republics. This may be associated with a previously low population of the final hosts (seals) at the main spawning grounds of this Baltic fish stock. During the latest decade, however, the Baltic has been invaded successfully by grey seals (*Halichoerus grypus*) and in 2013 more than 400 grey seals were colonizing the small rocky islets Ertholmene located east of Bornholm in the Southern Baltic. Cod caught in the area in 2011 and 2013 were investigated for the presence of nematode larvae in the flesh using a combination of artificial digestion (based on pepsin and hydrochloric acid) and compression techniques. Recovered parasites were diagnosed with molecular and classical techniques. It was found that up to 20% of the investigated cod samples were infected by third stage larvae of Pseudoterranova decipiens. The intensity (below five parasites per fish) was still low compared to other marine areas. The emerging infections of cod in the Baltic should be considered a risk not only to the local cod population in itself but also to the economy of the fish processing industry. Further, the zoonotic potential of these anisakid larvae may affect consumer health. Therefore the risk for further propagation and spreading of worms should be noted and seal control strategies implemented.

### O.6.3.2.002

**Absence of anisakid larval stages in body cavity and musculature of Danish, maricultured rainbow trout (*Oncorhynchus mykiss*)**

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INTRODUCTION Fish cultured in net cages are raised on heat treated pelleted feed and are excluded from participation in complex parasite life cycles occurring in the surrounding marine environment. The risk of infection with parasites transmitted through predation is therefore accordingly low under these circumstances. However, food web transmitted parasite infections may be passed on to cultured fish stocks via small fishes entering the net cage or invertebrate fauna associated with the net itself. The present investigation evaluated the food hygienic standard of rainbow trout from Danish mariculture regarding infection with zoonotic nematode larvae of the family Anisakidae.
MATERIAL AND METHODS During slaughter in November and December 2012, 100 rainbow trout (10 fish from each of 10 Danish mariculture facilities) were examined for anisakid larvae by macroscopical inspection of the body cavity (all peritoneal surfaces) and pepsin digestion of the belly flap musculature. The stomach contents were examined in order to assess the risk of parasite transmission.
RESULTS Half of the sampled trout ($n$ = 50) ranged from 0.383 to 1.168 kg (total body weight) and were characterized by pale or poorly colored flesh and no or limited fat deposits in the abdomen indicating a low intake of pelleted feed. The other half of the sample ($n$ = 50) ranged from 1.216 to 3.782 kg (total body weight) and had colored flesh and medium to heavy fat deposits indicating a high intake of pelleted feed. The stomach contents showed that 26% of the smaller trout had eaten molluscs, crustaceans and small fishes as opposed to 18% of the larger trout. Despite these findings, all trout were free from nematode larvae in peritoneum and belly flap musculature.
CONCLUSION Rainbow trout from Danish mariculture holds a high food hygienic standard as regards infection with larval stages of zoonotic, anisakid nematodes.

### O.6.3.2.003
**Imported zoonotic fishborne trematodes with ornamental fish – a case study from Denmark**

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Fishborne zoonotic trematodes are regarded as a serious health problem in countries where ingestion of dishes based on raw or under-cooked fish products is common. This tradition is being introduced in the industrialized part of the world including Europe. It is therefore relevant to report a recent introduction into Denmark of ornamental fish (*Xiphophorus maculatus*) heavily infected with exotic metacercariae of *Centrocestus* sp. A Danish company imported a batch of fish from Singapore but complained subsequently about lethargic and morbid fish despite several attempts of treatment for common diseases. We subjected the fish to a full parasitological examination and found heavy infections with *Centrocestus* sp. metacercariae (100% prevalence). Diagnosis was performed using molecular and classical parasitological techniques. The risk for spread of this and related diseases in European waters is discussed in line with the veterinary procedures for border inspection of imported exotic fish species.

## 6.3.3 Intestinal protists – diagnostic tools and emerging trends

### O.6.3.3.001
**Molecular epidemiology and subtype susceptibility patterns for *Blastocystis* from Sydney, Australia**

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Blastocystis is the most common enteric parasite isolated from human stools and has a world-wide distribution. There has been much controversy over whether this protozoan is considered pathogenic in humans but many recent *in vivo* and *in vitro* studies strongly suggest that this organism is a pathogen. It has been suggested that the pathogenicity of this parasite could be directly related to subtype infection. Due to the lack of knowledge on pathogenicity, treatment for this parasite varies with different susceptibility patterns being reported. This study included 510 stool samples submitted to St.Vincent's Hospital, Sydney Australia over a 10 month period. Stools were submitted to PCR and sequencing for subtype distribution. Isolates from four subtypes (ST) – ST1, ST2, ST3 and ST4 were then tested against a variety of antibiotics to determine susceptibility patterns. There was a 19% incidence rate of Blastocystis seen in the Sydney population. There were six different subtypes identified (1, 2, 3, 4, 6 and 8). Subtype 3 was the predominant subtype found in clinical samples with 45% of isolates belonging to this group. Metronidazole was found to be the least effective drug against all of the subtypes with paramomycin and ivermectin showing higher rates of sensitivity. This is the first large scale molecular epidemiological study on Blastocystis from Sydney, Australia. It was shown that ST3 is the most common subtype found in the Sydney population and symptoms could be subtype related. This study also highlighted that the most common treatment for Blastocystis, metronidazole, is not the most effective treatment option.

### O.6.3.3.002
**Genotype classification of *Blastocystis hominis* isolates from subjects with and without irritable bowel syndrome**

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Background Pathogenicity due to different Blastocystis species is controversial due to its heterogeneous antigenic and genetic characters within different geographic regions. The role of *B. hominis* in irritable bowel syndrome was demonstrated by its frequent significant detection in IBS patients, which requires more investigations to identify the genotypes of *B. hominis* clinical isolates obtained from different groups of patients; IBS, non-IBS GIT symptomatic patients and asymptomatic subjects, and to evaluate the infectivity and pathogenicity of the detected genotypes in experimental rats.
Methodology Three groups of *B. hominis* infected patients were included; IBS patients, non-IBS GIT symptomatic patients and asymptomatic subjects. Stool samples were cultured and parasite pellets were analyzed by PCR for determination of *B. hominis* genotypes. Experimental infection of rats was performed using different genotypes isolated from the study population. The histopathological lesions of the infected rats were assessed.
Results Genotyping of *B. hominis* isolated from different groups of patients revealed only three subtypes, 1, 2 and 3. Subtype 3 was the most common among the study population. All the subtypes were infective to rats. Subtype 1 was the most pathogenic as it was the only subtype that resulted in death of 25% of the animals. Histopathological intestinal examination of the infected rats revealed different findings among the groups regardless of the subtypes.
Conclusion The most dominant genotype was subtype three followed by subtype 1. No specific genotype correlated with pathogenic potential of *B. hominis* in any of the studied groups. Severity of pathological changes is not subtype-dependent suggesting that a variation of virulence exists between isolates of the same genotype.

### O.6.3.3.003
**Evaluation of enzyme immunoassay techniques for diagnosis of the most common intestinal protozoa in fecal samples**

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Objective This study was designed to evaluate antigen capture enzyme immunoassays (EIAs); Triage parasite panel and TechLab *E. histolytica* II in detecting Giardia lamblia, Cryptosporidium parvum and Entamoeba histolytica (*E. histolytica*) in faecal samples in comparison to microscopy, and in differentiating *E. histolytica* from *E. dispar*.
Methods Triage was evaluated with 100 stool specimens that were tested by the standard ova and parasite examination including staining with both trichrome and modified acid-fast stains. Differentiation between *E. histolytica* and *E. dispar* was performed using TechLab.
Results Microscopic examination revealed that 19% of the samples were positive for Giardia, 4% for Cryptosporidium, *E. histolytica*/*E. dispar* 1%, and other parasites 5%. By Triage, 23% of the samples were infected with Giardia, 5% with Cryptosporidium, and 2% with *E. histolytica*/*E. dispar*. Triage showed a sensitivity and specificity of 100% and 91.5%

respectively. The TechLab assay was negative with both samples diagnosed as *E. histolytica*/*E. dispar* by Triage, which suggested that they were *E. dispar*. Both tests showed no cross-reactivity with other intestinal protozoa.
CONCLUSION These results indicate that antigen detection by EIA has the potential to become a valuable tool capable of making stool diagnostics more effective.

## 6.4 Epidemiology, case studies, surveillance & information to the public

### O.6.4.001

**A cross-sectional prevalence study of gastrointestinal parasites in young and adult free-range chickens in El Sauce, Leon, Nicaragua**

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We conducted this cross-sectional study in free-range chickens in El Sauce, Nicaragua with the aim to determine the prevalence of gastrointestinal parasites. The study was carried out in two seasons, March representing the end of the dry season and November representing the end of the rainy season. A total of 448 young or adult birds were examined from 56 smallholder farms and necropsied to remove the gastrointestinal tract and trachea to collect the different parasite species. Four-hundred-and-forty-six birds were infected with nematode species, 411 with cestodes, one with acanthocephals and one with trematodes. Twenty-five different helminth species were identified (fifteen nematodes, six cestodes, one acanthocephela and one trematode). Each chicken harbored a mean of seven different helminth species. The most prevalent nematodes were Heterakis spp (92.%), Strongyloides avium (88.6%) and Ascaridia galli (85.5%) whereas the most prevalent cestodes were Raillietina spp (85.9%) and Hymenolepis spp (62.7%). The mean worm burden was 20.8 (range 0–389) worms per chicken. The distribution of parasites between hosts varied from a moderate overdispersion in the cases of *A. galli* and *H. gallinarum* to a heavy degree of overdispersion for Capillaria species. Significant differences between age groups and seasons were observed, and two examples will be mentioned here. In November the young chickens had a significantly higher prevalence and worm burden of *A. galli* compared to the adults. In the case of *Amebotaenia cuneata* no significant differences in prevalence nor burden could be detected between age groups in November, whereas both a significantly higher prevalence and burden was detected in the adults compared to the young age group in March. These differences are a reflection of the combined effect of different survival of eggs in the environment, seasonal variations in the abundance of intermediate hosts necessary for the transmission of several species and acquired immunity.

### O.6.4.002

**Longitudinal study of gastrointestinal parasites infection in free-range chickens in El Sauce, León. Nicaragua**

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This observational longitudinal study was carried out on free-range chickens on ten smallholder farms to understand the population dynamics of parasite infections in relation to age, rainfall and weight gain. A total of 188 free-range chickens were included from hatching and followed monthly by determination of body weight and faecal egg/oocyst counts until necropsy at 12 months of age. The birds were divided in two groups hatched either in March or April 2011. All chickens excreted coccidia oocysts on at least one sampling point and they also excreted helminth eggs in at least one sampling. Oocyst excretion began as early as 1 week of age. The first excretion of *A. galli*/*Heterakis* spp eggs was detected at the age of 1 month. Among the chickens hatched in March the median age at the first *A. galli*/*Heterakis* spp. egg excretion was 73 days whereas the birds hatched in April were naïve until a median of 56 days of age. The excretion of eggs of these species showed a bimodal pattern with the highest levels in July and February, which may reflect the age of the birds, rainfall (the rainy season lasted from May to November) and the time where the hens became mature and started laying. The effect of parasite burden on weight gain was determined by dividing the chickens into quartiles (Q1–Q4) according to helminth burden upon necropsy. The total weight gain was compared between quartiles by one-way ANOVA. A significant impact of worm burden on body weight gain could be determined only for *A. galli* ($P = 0.028$) where birds belonging to Q4 had a 11.2% lower weight gain than the other groups. Interestingly, no significant weight gain difference was observed between birds of Q1, Q2 and Q3, which may indicate a threshold effect of worm burden on weight gain.

### O.6.4.003

**Transmission dynamics of *Ascaris suum* in Danish organic pig farms**

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Ascaris suum is the most common nematode in pig farms, but the on-farm transmission dynamic is not well described. A 1-year field study on organic pig farms was therefore carried out, sampling every 4 months (September 2011–June 2012). The study mapped infection levels of animals (starters, finishers, dry and lactating sows), liver white spots at slaughter (105 finishers), egg contamination levels in pens (starters and finishers) and on pastures (dry sows, lactating sows and starters). Three different areas (resting, intermediate and latrine areas) were identified and sampled in each pen in deep litter housing systems (two farms) and shallow litter housing systems (three farms). All pen areas were contaminated with eggs and levels were similar in both housing systems, but generally higher (5–25 times) in latrines compared to the other two areas. Significantly higher numbers of larvated eggs were found in shallow litter (6.9%) compared to deep litter (0.4%; $P < 0.0001$). Most recovered eggs were not fully developed (i.e. infective), but laboratory embryonation of *A. suum* eggs isolated from bedding material revealed that 39–97% of the eggs were viable. The majority of pastures contained infective *A. suum* eggs. Averaged across farms and samplings, the *A. suum* prevalence in starters, finishers, dry sows and lactating sows was 48%, 64%, 28% and 15%, respectively. The highest egg counts were recorded for starters and finishers and 88% of finishers had liver white spots. In conclusion, piglets appeared to first become infected on the farrowing pastures and that exposure continued on weaning paddocks and inside the stables all the way up to slaughter. Highly infective bedding material should be stored, composted or treated before use and never used for pastures to limit contamination of environment with *A. suum* eggs.

## O.6.4.004

### Zoonotic parasite infections among Estonian veterinarians compared with general population

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Very little is known of the presence of zoonotic parasite infections in Estonia. Previous studies indicate that the Estonian population may be more commonly infected with zoonotic parasites compared to Nordic countries. Specific risk groups, such as veterinarians, are frequently in contact with potentially infected animals. The main aim of this study was to estimate the seroprevalence of the zoonotic parasites Echinococcus, Toxoplasma, Trichinella, Ascaris, Taenia and Toxocara in the Estonian population and veterinarians. Venous blood samples and questionnaire data were collected in autumn 2012 from 158 volunteering veterinarians at an annual conference in Tartu. Demographically balanced sample representing Estonian general population was acquired from Estonian Genome Centre ($n$ = 500). All samples were investigated for IgG antibodies against Trichinella, Taenia, Toxoplasma, Echinococcus, Toxocara and Ascaris using commercial enzyme linked immune-assay (ELISA) kits. Significance of differences between seroprevalences was estimated by two-sample z-test for comparison of proportions. The apparent seroprevalences among veterinarians and the general population were respectively: *Trichinella spiralis* 3.2% (CI 95% 0.5–5.9) and 8% (CI 95% 5.6–10.3), $P$ = 0.037; *Taenia solium* 0.6% (CI 95% −0.6–1.8) and 2.2% (CI 95% 0.9–3.4), $P$ = 0.189; *Toxoplasma gondii* 46.2% (CI 95% 38.4–54) and 56.4% (CI 95% 52.1–60.1), $P$ = 0.025; *Echinococcus* spp. 4.4% (CI 95% 1.2–7.6) and 1.4% (CI 95% 0.4–2.4), $P$ = 0.023; *Toxocara canis* 10.1% (CI 95% 5.4–14.8) and 15% (CI 95% 11.9–18.1), $P$ = 0.120; *Ascaris lumbricoides* 17.7% (CI 95% 11.8–23.7) and 26.6% (CI 95% 22.7–30.5), $P$ = 0.023. The preliminary data show a higher Echinococcus seroprevalence in veterinarians compared to the general population, while having lower seroprevalences for other zoonotic parasites. Still, all apparent seroprevalences of investigated parasites seem generally to be higher in Estonia than in Nordic countries.

## O.6.4.005

### Endoparasites in Eastern European stray dogs imported to Norway

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INTRODUCTION Harmonization of regulations in the European Union (EU) and European Economic Area (EEA), as of 1st January 2012, has led to an increase in the number of stray dogs imported to Norway from Eastern European countries, notably Romania. The entry requirements for dogs entering Norway are rabies vaccination and prophylactic Echinococcus multilocularis treatment.

MATERIAL AND METHODS The criteria for inclusion in the study were that the dog was considered a stray animal in its country of origin, and had arrived from Romania, Hungary, the Balkan and Baltic countries during 2012. Blood samples from 75 and fecal samples from 68 dogs were submitted to the Norwegian Veterinary Institute. Faeces were examined by McMaster's method, direct smears and Baermann's method for parasite eggs, oocysts and larvae. Blood samples were examined for Dirofilaria immitis surface antigens using a *D. immitis* Ag-ELISA, while IFAT was used to detect antibodies against Babesia canis.

RESULTS Eggs of *Uncinaria stenocephala* (5.9%), Trichuris sp. (11.8%), *Giardia* sp. (15.8%), *Isospora* spp. (2.9%), *Toxocara canis* (1.5%) and *Toxascaris leonina* (2.9%) were identified in faecal samples. Antibodies against *Babesia canis* were detected in 9.6% of the blood samples. Antigens from adult female *Dirofilaria immitis* worms were detected in 8.2% of the examined dogs.

CONCLUSION The parasites detected only occur at a very low to low prevalence or not at all in native Norwegian dogs. We conclude that the import of stray dogs represents a real risk of spreading pathogens across borders. This risk should not be neglected; several of the parasites in question, once introduced, may become endemic and pose a threat to both animal and human health. Education of dog owners about the risks involved with import and about risk reducing measures is essential.

## O.6.4.006

### Reporting the occurrence of the mosquito-borne filarial nematode: setaria tundra in three roe deer (*Capreolus capreolus*) in different localities in Denmark

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Setaria tundra is a filarial nematode that is transmitted between several species of angulates through mosquitoes. Infections with *S. tundra* were previously described in European countries, including Fennoscandinavia. Setaria tundra inhabits the abdominal cavity of reindeer and is generally considered harmless but severe morbidity and mortality for both reindeer and moose were recently reported in Finland. In this report, worms of *S. tundra* were recovered from three deer, one hunted in October 2010 in the eastern part of peninsular Jutland, a second deer was hunted in May 2011 in the south-west of the island, Zealand, and the third deer was hunted in May 2012 in the southern part of Zealand. The worms were identified as *S. tundra* based on morphology and/or molecular typing of the mitochondrial 12S rRNA and cox1 genes. Roe deer are generally considered asymptomatic carriers of *S. tundra*, and the recovery of the worms indicates the presence of this parasite that is of high concern to breeders of roe deer and other ungulates. This parasite may have been present but overlooked. Previous outbreaks of setariasis in Scandinavia have been associated with marked climatic changes, such as unusually warm summers. Given the right circumstances, the parasite has demonstrated capacity to a dramatic spread. The presence of highly populated deer farms may also enhanced the spread of this parasite. These facts highlights the importance understanding the ecological factors that might promote the expansion of this nematode may as well help to predict disease outbreaks of other filarial nematodes that utilize the same vectors.



 

## 6.5 Parasites – treatment and control

### O.6.5.001

**Differences in *in vitro* sensitivity and accumulation of anthelmintics in *Trichuris suis* and *Oesophagostomum dentatum***

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INTRODUCTION The current strategy in control programs targeting the whipworm of man (*Trichuris trichiura*) is administration of a single-dose albendazole (ALB, 400 mg) or mebendazole (MBD, 500 mg). Both reported as having non-satisfactory efficacy against *T. trichiura*. *Trichuris suis* and Oesophagostomum dentatum are both found in the large intestine of pigs, but in contrast to *T. suis*, *O. dentatum* is highly sensitive towards benzimidazoles (BZs).
MATERIAL AND METHODS We used *T. suis* as a model for *T. trichiura*, to evaluate whether the purported difference in sensitivity towards BZs could be related to different levels of accumulation of BZs within the two parasitic species. A motility assay was used to evaluate the efficacy of fenbendazole (FBZ), ALB and levamisole (LEV) *in vitro* on adult *T. suis* and *O. dentatum*. High performance liquid chromatography (HPLC) was used to measure the concentrations of FBZ, ALB and LEV within dead and alive worms and to estimate the maximum binding of FBZ and ALB within the two species.
RESULTS The motility of *T. suis* was less affected by increasing concentrations of FBZ ($P < 0.028$) and ALB ($P < 0.0001$) than *O. dentatum*, whereas LEV decreased the motility of *T. suis* more than *O. dentatum* ($P < 0.0002$). The concentration of ALB and LEV was significantly lower in *T. suis* than in *O. dentatum* when adults of both were alive (ALB: $P = 0.003$, LEV: $P = 0.02$) and dead (ALB: $P = 0.002$, LEV: $P = 0.008$), but the concentration of FBZ was only lower in *T. suis* when worms were dead ($P = 0.003$). Maximum binding of FBZ and ALB was significantly lower in *T. suis* than *O. dentatum* ($P < 0.0001$, $P = 0.002$ respectively).
CONCLUSION Our data suggests that differences in efficacy of FBZ and ALB against *T. suis* and *O. dentatum in vitro* can be related to differences in the uptake of drugs.

### O.6.5.002

**Efficacy of forage chicory, tannins and praziquantel against metacestode stage of *Hymenolepsis diminuta* (Cestoda) in the *Tenebrio molitor* (Coleoptera) an invertebrate – parasite model**

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Invertebrate models have several advantages over vertebrate models, with regards to ethical clearance, legislation, time and economy. In the present scenario of developing resistance against chemical anthelmintics, bioactive plant products could be an effective alternative for treating parasite infections. Previous studies have shown that the extracts from forage chicory (*Cichoriumintybus* L.), and plants rich in condensed tannins have negative effects on nematode development, however the effects against other helminth taxa are virtually unknown. The present *in vitro* study was therefore conducted to assess the effects of chicory extract and tannins on *Hymenolepsis diminuta* cysticercoids isolated from the intermediate host *Tenebrio molitor* (Coleoptera), in comparison to the well-known taenicide praziquantel. Beetles were inoculated with infected rat feces containing *H. diminuta* eggs. After 2 weeks of incubation, cysticercoids were isolated from the beetles and treated with 150 $\mu$l of either forage chicory extract (5, 1 or 0.2 mg/ml), tannins (5, 1 or 0.2 mg/ml), praziquantel (0.01, 0.001 or 0.0001 mg/ml), Dimethyl sulfoxide (DMSO 2%), or Milli-Q TM water for 1 h. The inhibitory effect of chicory and praziquantel was measured as a proportion of excystation of cysticercoids in relation to the DMSO 2% (control) and tannins with Milli-Q TM water (control). Praziquantel had a dose-dependent inhibitory effect on cysticercoids excystation. Chicory and tannins had also inhibitory effect, however higher concentration was required compared to the praziquantel. At similar concentrations, tannins had a higher inhibitory effect than chicory. Our results therefore indicate that chicory extract and in particular tannins may have anthelmintic activity against the metacestode stage and that the *H. diminuta*-T. molitor model demonstrates similar effects of praziquantel compared to what is observed in vertebrate models.

### O.6.5.003

**Direct anthelmintic effects of purified Chicory extract against *Ascaris suum* and *Oesophagostomum dentatum***

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INTRODUCTION New methods of helminth control are urgently needed in livestock production. Resistance to conventional anthelmintic drugs and consumer demand for organic animal products have made reliance on existing drugs unsustainable. *Ascaris suum* and *Oesophagostomum dentatum* are the two most prevalent helminths in pig production, both on organic farms and increasingly also in conventional production systems. Chicory (*Cichorium intybus*) has shown potential to be used as a natural deworming agent, although the mechanisms involved remain unclear. Here, we investigated whether purified extracts of chicory could directly kill these parasites *in vitro*.
MATERIALS AND METHODS Fresh chicory leaves (cv. Spadona) were collected from an organic farm in Denmark, stored frozen and then extracted with methanol. Further purification was performed by solid-phase extraction to enhance recovery of secondary metabolites. The extract was then resuspended in 2% DMSO. *A. suum* larvae were obtained by artificially hatching fully embryonated eggs *in vitro*. Anthelmintic activity was assessed using a modified agar-migration inhibition assay. *O. dentatum* eggs were obtained from mono-infected donor pigs and used in a standard larval development assay. Negative and positive controls consisted of 2% DMSO and ivermectin, respectively.
RESULTS Chicory had a marked anthelmintic effect against both parasite species. After incubation with the chicory extract at concentrations of 500 or 250 $\mu$g/ml, *A. suum* migration was inhibited by 95%. At the lowest concentration tested (32 $\mu$g/ml), migration was still inhibited by >50%. Chicory also inhibited the development of *O. dentatum* eggs to infective larvae, with 100% inhibition at concentrations of $\geq$250 $\mu$g/ml, and reduced larval development at lower concentrations.
CONCLUSIONS Extracts of chicory have direct anthelmintic effects against two important parasites of pigs. This provides further evidence of the role that chicory may play as an

alternative helminth-control agent. Further experiments are ongoing to determine the active compounds in the extract.

### O.6.5.004

#### *In vitro* evaluation of anthelmintic activity of various tannin structures against *Cooperia oncophora*

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To prevent the spread of resistance among gastro-intestinal nematode populations, the use of bioactive tannin-rich plants is currently investigated as an alternative to the exclusive use of anthelmintic (AH) synthetic drugs. Studies of AH effects on cattle nematodes using tannin-rich legumes such as *Onobrychis viciifolia* have been carried out *in vitro* but the contribution of all structural parameters from their condensed tannins has not yet been completely elucidated. The aim of this study was to investigate the relationship between structure and AH activity *in vitro*. A series of condensed tannins extracts and fractions from various plants was chosen according to their monomeric composition (prodelphinidin/procyanidin ratio) and additionally characterised for their degree of polymerisation (mDP) and cis/trans ratio by thiolytic degradation. These tannins were examined for their AH activity against *Cooperia oncophora* using the Larval Feeding Inhibition Assay (LFIA) and the Larval Exsheathment Inhibition Assay. Preliminaries results from the LFIA showed a positive correlation between AH activity and increasing prodelphinidin/procyanidin and cis/trans ratios of the tested tannins, respectively. The variation in mDP between plants was very limited and the influence on AH activity could not be assessed. These findings should be confirmed by further *in vitro* assays including others tannin fractions. Moreover, hazelnut skin crude extract presented a total inhibition of larval exsheathment at 600 $\mu$g/ml suggesting that some agro-industrial resources may be helpful in sustainable control of cattle nematodes.

## 6.6 Molecular parasitology and evolution

### O.6.6.001

#### Molecular characterization of *Haemoproteus minutus* (Haemosporida, Haemoproteidae), a virulent parasite of birds

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Avian malaria parasites and related haemosporidians are important agents of diseases in domestic and wild bird species. For many reasons, the observation of direct negative influence of haemosporidians on wild free-living birds is difficult; however, the reports about death of captive birds due to haemosporidioses are increasingly frequent, especially in aviaries and zoos. Recently, the lineage hTURDUS2 of *Haemoproteus minutus* (Haemosporida, Haemoproteidae) was determined using molecular methods from tissue samples of captive parrots died in aviaries in Europe. This parasite lineage is naturally widespread and prevalent in the blackbird *Turdus merula* throughout its entire distribution range of this bird. Species identity of other closely related haemoproteid lineages, which have been recently reported in dead parrots, remains unclear, but it is important to determine them for better understanding of the epidemiology and pathology of avian haemoproteosis. Using polymerase chain reaction (PCR)-based and microscopic methods, we analysed 265 blood samples collected from 52 species of wild birds in Eurasia. Single infections of the lineages hTURDUS2, hTUPHI1 and hTUCHR01 were detected. We identified species of these haemoproteids based on morphology of their blood stages and conclude that these lineages also belong to *H. minutus*, a widespread parasite of different species of thrushes (genus Turdus), whose serve as reservoir hosts of this haemoproteid infection. Based on the recent literature data about mortality caused by *H. minutus* in captive parrots in Europe, we consider that this widespread parasite and other phylogenetically closely related species are worth more attention in veterinary medicine as possible underestimated agents of haemoproteosis in non-adapted birds. Molecular markers are particularly valuable in detecting and typing haemosporidian tissue stages, which are similar in many parasite species and hardly can be identified to species level based on the parasite morphology.

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### O.6.6.002

#### Gene expression in mesenteric lymph nodes of pigs with two different genotypes associated with resistance or susceptibility to *Ascaris suum*

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Introduction In pigs, two single nucleotide polymorphisms (SNP TXNIP and SNP ARNT), both on chromosome 4, have been reported to be associated with roundworm (Ascaris suum) burden.
Materials and Methods In the present study, we selected pigs with two TXNIP genotypes (AA; $n = 24$ and AB; $n = 24$. Pigs were trickle-infected with *A. suum* from 8 weeks of age until week eight post first infection (PI) when the pigs were slaughtered. An uninfected control group (AA; $n = 5$ and AB; $n = 5$) was also included. At necropsy, we collected lymph nodes for expression studies and recorded worm burdens (macroscopic worm burden and total worm burden) and faecal egg counts. Gene expression in mesenteric lymph nodes was evaluated by qPCR using a microfluidic-based platform. We included markers of different T cell responses (e.g., TH1-type response, TH2-type response, regulatory T cells) as well as different candidate genes in linkage disequilibrium with the SNP TXNIP marker.
Results Data indicated that pigs of the AA genotype were resistant to infection and AB pigs were susceptible. Twenty-seven genes passed quality testing and were included in the gene expression analysis. Preliminary data analysis using a mixed model with genotype and infection status as fixed effects showed that CCL17 ($P < 0.05$), IL4 ($P < 0.05$) and TGFB1 ($P = 0.055$) were more abundantly expressed in *A. suum*-infected pigs than


 

in the control group. Interestingly, the expression of IL13 was higher in pigs of the susceptible AB genotype than in pigs of the AA genotype ($P = 0.064$). A mixed model with genotype and total worm burden as fixed effects showed a significant correlation between relative expression level and total worm burden for CCL2 ($P < 0.05$), IL13RA2 ($P < 0.01$), NOS2 ($P < 0.01$) and STAT6 ($P < 0.01$). Further analyses of qPCR data will reveal some of the key genes involved in the pig's response to *A. suum* infection.

### O.6.6.003

#### Challenges of genomic DNA isolation from parasites inhabiting nucleated red blood cells

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Avian haemosporidian parasites (Apicomplexa: Haemosporida), including the agent of malaria (gen. Plasmodium) are prevalent in Europe up to the Northern Polar Circle. Until now, the majority of bird haemosporidian parasite molecular studies have used mitochondrial genes. The lack of genomic material from haemosporidians has prevented the construction of primers for amplification of rapidly evolving genomic regions. These challenges are determined by the structure of avian red blood cells. Unlike mammals, bird and reptile red blood cells possess nuclei, resulting in big amount of host DNA in each blood sample. Another relevant problem in molecular studies of avian haemosporidians is deciphering the haplotypes of parasite species during co-infections, which are prevalent in wildlife. We developed (i) a new method that generates large amount of purified avian haemosporidian DNA and (ii) a technique for isolation of single cells for these intracellular parasite molecular studies. The first method is based on *in vitro* exflagellation and development of numerous microgametes, with their subsequent separation using simple centrifugation; the obtained template was used for whole genome amplification. The second method is based on the laser microdissection microscopy; we used the precise UV-laser cut of parasite cells. Single cells of haemosporidians were dissected and placed in separate micro-tubes; DNA was extracted, fragments were amplified and sequenced for genomic analysis. Our results reveal that this purification method provides opportunities to collect pure parasite template for DNA studies, which can be used for various genetic analyses, including whole genome sequencing. We also show that the single cell dissection using laser microscopy can be applied for parasite species molecular identification during co-infections or other purposes where genomic information is needed on the level of individual cells.

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## 6.7 Parasite ecology

### O.6.7.001

#### Comparative performance of two *G. salaris* strains on salmon and charr under comparable common garden conditions

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*Gyrodactylus salaris* is highly pathogenic to Norwegian salmon. However, strains have been isolated which lack infectivity to salmon and appear to normally infect other salmonid hosts. We describe the performance of the Pålsbu *G. salaris* strain, isolated from charr (*Salvelinus alpinus*), and compare it with the performance of the Lierelva strain, pathogenic to salmon. The Pålsbu strain shows low infectivity for Numedalslågen salmon. The Lierelva strain nonetheless, grows exponentially on this stock, confirming its susceptibiity to a salmon-infecting strain of the parasite. Only one third of Pålsbu strain infections ($n = 33$) became established on salmon and none surpassed 40 individuals. However, the parasite persisted for up to 12 weeks. On Alta and Svalbard charr stocks, both strains of parasites became established and populations grew successfully. Nevertheless, whereas infections of Lierelva parasites peaked after 3 weeks at a mean size of 24 worms, those of Pålsbu parasites grew more slowly with smaller sizes and peaked after 5–6 weeks. It was impossible to maintain the Pålsbu strain on salmon as too few infections became established. On charr, Lierelva parasites had higher rate of reproduction, giving birth approximately 1 day earlier for second, third and fourth births than Pålsbu parasites. Surprisingly, the Lierelva parasites showed identical fecundity and reproductive rate when maintained on salmon, and age-specific mortality was identical on the two hosts. We conclude that (i) no cost to infection of charr strains by the salmon-infecting forms of *G. salaris* exists, apart from the effect of the host response. However, charr could act as a natural host for salmon-infecting strains of *G. salaris* and (ii) the Pålsbu strain of *G. salaris* has a slightly reduced reproductive rate relative to the Lierelva strain, even on its normal host. It appears also to have some behavioural mechanism which prevents it from becoming established on salmon.

### O.6.7.002

#### Disentangling density dependence and time dependence in infections of *G. salaris* on salmon

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*Gyrodactylus salaris* presumably grows without check until susceptible salmon hosts die. However, parasite populations are subject to growth constraints. Using linear modelling (LM and GLM in R) and bayesian (WinBugs) approaches we re-analysed old data sets for growth of *G. salaris* on individually isolated salmon. This allows realistic reconstruction of population growth rates and reinforces evidence that it is impossible, on existing datasets, to identify differences in parasite population growth between individual salmon. There is evidence of density dependence in *G. salaris* populations on western Norwegian salmon stocks, such that population growth slows as the parasite population grows, due to nutritional or space limitation. In such stocks, parasite population growth rates show negative linear or slightly concave relationships with parasite population size which are explained by density dependence. On eastern Baltic Neva fish, density dependence also occurs, but the relationship between parasite population size and growth rate is strongly convex. This appears to represent a time-dependent effect, presumably a host response. Other stocks show intermediate performance. The Scottish Conon and Shin stocks, strongly suggest the onset of a time-related response after 30–40 days. A surprising finding comes from re-analysis of the Indalselv Swedish stock, which appears to lack any evidence of a host response, but does show density dependence. This limitation of growth appears to be due to a poor growth rate from the beginning. These analyses suggest that a range of mechanisms exist to limit *G. salaris* population growth on salmon

stocks. Highly susceptible Norwegian stocks may be restricted to the West and North West coast watersheds where the disease has had the greatest impact. Morover, considerable variation exists between different replicates within the same stock of fish. This suggests that a possible 'environment X genotype' interaction can affect parasite population growth rate and the final outcome of infections.

### O.6.7.003

**Changes in establishment rates during the time course of a continuous *Ascaridia galli*-infection in chickens, using a genetic marker**

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This study investigated the changes in establishment rates during the time course of a 6-weeks trickle infection of chickens with *Ascaridia galli* with two different egg cohorts (type a and type b) at two dose levels. These cohort specific egg batches were harvested from the uterus of female worms of two different haplotypes and were genetically identified using PCR-RFLP on the cox1 gene in the mitochondrial genome. Fifty-six, 8-weeks-old Lohmann Brown Lite chickens were divided into seven groups. The infectivity of the two egg cohorts was tested by infecting two groups of chickens with 500 eggs of either cohort. At slaughter 3 days post infection no significant difference in the infectivity was observed between the two cohorts ($P = 0.6$). Further, group ab100 was trickle infected for 3 weeks with 100 eggs of cohort a (twice weekly) followed by eggs of cohort b for another 3 weeks. Group ba100 was treated accordingly but in the opposite order (cohort b preceding a). A similar infection regime was applied for group ab25 and ba25 but with a lower inoculation dose (25 eggs per bird twice weekly). The rest group was left as uninfected control. All the birds of these five groups were killed 2 weeks after the last inoculation (week 8). It was found that the establishment of the late infection was dose dependent. In lower dose groups both the early and late infections established equally well (48–56%), whereas in the high dose groups the early infection was recovered in higher proportion (83–87%) than the late infection irrespective of genetic cohorts. This result indicates the presence of dose dependent resistance against reinfection after 3 weeks of continuous infection, and this resistance seems to act by reducing the establishment of later infections rather than by affecting the already established parasite population.

### O.6.7.004

**The role of *Toxoplasma gondii*-produced tyrosine hydroxylase (TgTH) in parasite manipulation of host behaviour**

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The predilection of *Toxoplasma gondii* cysts for the brain of its intermediate rodent host places it in a privileged position to manipulate host behaviour through a variety of mechanisms, increasing the chance of successful transmission to its definitive feline host. Using the epidemiologically and clinically applicable rat-*T. gondii* model, and incorporating a range of both novel and classical non-invasive behavioural and physiological assays, our overall prediction was that the parasite is manipulating its host, at least in part, via *T. gondii*-produced tyrosine hydroxylase (TgTH), an enzyme involved in the synthesis of various neuromodulators including L-DOPA, dopamine, and noradrenaline. Preliminary results indicate that TgTH may be involved in increasing activity levels within the host, but may not be exclusively involved in the specific response to feline odour observed in infected rats. There may be evidence that TgTH influences the host via elevated noradrenaline levels. Gender-specific effects of *T. gondii* are seen which involve interactions between infection, gender and potentially testosterone levels. These initial results support the hypothesis that TgTH plays a part in the mechanism involved in behavioural alterations that may facilitate parasite transmission, but that additional mechanisms of action may be involved in the specific 'fatal feline attraction' to the parasite's definitive host. This is further evidence for the complexity of the evolution of parasite manipulation within the mammalian brain, which is likely to involve multiple pathways to maximise transmission success. These results will be discussed in terms of their theoretical and applied implications.

## 6.8 Methods in parasitological research

## 6.8.1 Geospatial Health: Mapping and modelling parasitic infections

### O.6.8.1.001

**Exploring patterns and distributions of parasite intermediate host snails in Africa using climate-based species distribution modelling**

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INTRODUCTION Like any other species, many parasitic diseases have 'natural habitats': they are found in focal areas where the spatial distribution of the parasite, host, vector and required environmental (e.g. climate) conditions coincide. Climate is considered to be the predominant range-determining mechanism at large spatial scales, but species' ranges may be controlled by other environmental or non-environmental factors. In disease risk mapping, often the goal is merely that of prediction, and the linkage between modelling practice and ecological theory are often weak. In particular, the consideration of the ecology of the vector and intermediate host species, by some considered the prerequisite for successful disease elimination and eradication programmes is often missing.
MATERIALS AND METHODS Here, the environmental drivers of the distribution of several freshwater snail species in Africa is explored to obtain a deeper understanding of the eco-epidemiology of the snail-borne parasitic infections they host. Climatic and non-climatic variables are associated with extensive snail distribution data in a species distribution modelling framework, using a maximum entropy method (Maxent), often used by ecologist and macro-ecologists. In particular, the distributional patterns and determinants of the intermediate host snail species of parasites causing human schistosomiasis (Biomphalaria spp and Bulinus spp), are investigated.
RESULTS AND CONCLUSIONS Maps of the distribution of schistosome intermediate host snail across Africa are presented based on identified relationships with environmental predictors. Models that included a combination of climatic and habitat


 

related predictors, had better predictive ability compared to models based on either climate or habitat-derived variables alone. Importantly, even though climate per se matters for the distribution, the impact of humans on habitat plays a crucial role in determining the distribution of the intermediate host snails in Africa.

### O.6.8.1.002

**Mapping and modeling *Dirofilaria* infections in Europe**

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Climatic changes, together with an increase in the movement of dogs across Europe, have caused an increase in the geographical range of *Dirofilaria immitis* and *D. repens* infections. A Geographic Information System based on thermal regimen was constructed to identify areas potentially suitable for *Dirofilaria* transmission in Europe. These models are based on evidence that: (i) there is a threshold of 14°C below which *Dirofilaria* development will not proceed in mosquitoes; (ii) there is a requirement of 130 growing degree-days for larvae to reach infectivity, and; (iii) there is a maximum life expectancy of 30 days for a mosquito vector. The output of these models predicted that the summer temperatures (with peaks in August) are sufficient to facilitate extrinsic incubation of *Dirofilaria* even at high latitudes. Recently, an additional model was constructed to verify the influence of temperature in the course of three decades (1980–1989, 1990–1999 and 2000–2012) on the risk of infection by *Dirofilaria* in Italy. The results showed an expected increasing trend of temperatures, an increase of the *Dirofilaria* generation numbers into the mosquitoes and a significant extension of the infection risk from 5–6 months (1980–1989) to 6.5 months (1990–1999), up to more than 7 months (2000–2012). These findings show that geospatial tools are very useful for mapping, monitoring, forecasting and surveillance of both heartworm and subcutaneous dirofilariosis.

### O.6.8.1.003

**Epidemiological and spatiotemporal features of acute schistosomiasis in the People's Republic of China**

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BACKGROUND Despite significant reduction during the past 50 years, data suggest that schistosomiasis has re-emerged in China recently. Therefore, the national control program revised its strategy in 2004.
METHODS Surveillance data on acute schistosomiasis and outbreaks were extracted from the National Diseases Reporting Information System (NDRIS) from 2005 to 2012. The data was diagrammatized and analyzed using SPSSv17.0. The spatiotemporal cluster features were described using a discrete Poison model in SatScan V9.1.1, and ArcGIS 9.3.
RESULTS A total of 1047 cases were extracted from the NDRIS. 989 (94.5%) cases were reported from the lake and marshland regions (Hunan, Hubei, Jiangxi, Anhui and Jiangsu province), only 55 (5.3%) in the mountainous regions (Sichuan, Yunnan province) and 3 (0.3%) were imported from other countries. The number of annual cases declined significantly from 2005 to 2012 by 97.7%. Most cases were school-age children, farmers and fishermen. Only five outbreaks were reported in Hubei Province and one in Sichuan Province in 2005, and none in subsequent years. Totally, 85 cases were recorded as infected in other provinces. The cases clustered from June to November and a total of nine clusters were detected with a higher risk in the lake and marshland regions of Hunan, Hubei, Jiangxi, Anhui and Sihchuan provinces.
CONCLUSIONS Decreasing numbers of cases from 2005 to 2012, indicate that the revised control strategy has played a role in reducing the prevalence of schistosomiasis. However, potential risk of infection and/or re-emergence remains in the lakes and marshland regions.

## 6.10 Role of natural IgM binding in parasitic diseases

### O.6.10.001

**The role of non-specific IgM binding to a *Plasmodium falciparum* protein PfEMP1 which mediates rosetting**

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INTRODUCTION The pathogenicity of *Plasmodium falciparum*, which is responsible for most malaria-related severe morbidity and essentially all the mortality, is related to the capacity of *P. falciparum* -infected erythrocytes (IEs) to adhere to vascular host receptors. This adhesion is mediated by members of the PfEMP1 family of clonally variant *P. falciparum* erythrocyte membrane proteins. Expression of the relatively conserved PfEMP1 protein VAR2CSA mediates IE adhesion to placental CSA. It has been proposed that acquisition of protective immunity to VAR2CSA is delayed by its affinity for non-specific IgM, which could interfere with specific IgG recognition of the antigen. In addition to VAR2CSA, a number of PfEMP1 proteins that mediate rosetting also bind non-specific IgM. We therefore speculated that non-specific IgM binding to rosetting isolates serves the same immune-evasive purpose as in the case of VAR2CSA.
MATERIALS AND METHODS We used recombinant proteins representing individual domains and the entire extracellular part of the rosetting PfEMP1 protein, HB3var06 to study this hypothesis.
RESULTS AND CONCLUSIONS We could map the IgM binding region to the C-terminal part of the molecule, which corresponds well to the location of the binding site for non-specific IgM binding in VAR2CSA. However, SAXS analysis showed that full-length HB3var06 has an elongated tertiary structure rather than the globular conformation of VAR2CSA. We used domain-specific antisera to study the impact of this structural difference on the ability of non-specific IgM to interfere with binding of

antigen-specific IgG, and the reason for the previously described link between rosetting and IgM binding.

### O.6.10.002
**Discovery of novel IgM binding PfEMP1 variants in *Plasmodium falciparum* line NF54**

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Introduction The antigen family *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) mediates binding of infected erythrocytes (IEs) to a variety of human receptors. The IE binding phenotype depends on which PfEMP1 variant is being expressed. Each parasite genome contains about 60 var genes encoding different PfEMP1 variants. This and the substantial interclonal var gene diversity together make the repertoire of PfEMP1 variants immense. PfEMP1 variants involved in placental malaria (VAR2CSA) and some variants displaying the rosetting phenotype (shown to be correlated with severe childhood malaria) are able to bind nonspecific IgM. We have previously shown that this binding results in masking of protective IgG epitopes on VAR2CSA-positive IEs. In order to determine if this apparent immune evasion is a general property of IEs expressing IgM-binding PfEMP1 variants, and to map the IgM binding epitope, we set out to identify new IgM-binding PfEMP1 variants in the genetically well-characterized *P. falciparum* line NF54.
Material and Methods Switching among transcription of different var genes rarely occurs in long-term *in vitro* cultures, probably as a reflection of epigenetic memory. Therefore, the selection of IEs expressing particular PfEMP1 variants can be troublesome. We overcame this problem by establishing a novel method based on the NF54 clone G6 that had its epigenetic memory deleted by the plasmid VBH. This clone was used in flow cytometry assisted single-cell sorting experiments to select for IEs binding unspecific IgM.
Results and Conclusion So far, we have identified two additional PfEMP1 variants in NF54 (PFL0020w and MAL6P1.4) that bind unspecific IgM. Furthermore, we have further defined the binding site for IgM to the C-terminal part of IgM-binding PfEMP1 variants. These results pave the way for a more comprehensive analysis of the functional significance of the binding of non-specific IgM that is characteristic of some, but by far all, PfEMP1 variants.

## 6.11 Parasite immunology and pathology

### O.6.11.001
**The impact of induced shift in TH1/TH2 balance on cruise of *Trichobilharzia* infection**

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Bird schistosomes of *Trichobilharzia* spp. are able to infect mammals including human as non-specific (abnormal) hosts; experimental infections lead to partial development of larvae (schistosomula) which migrate through several tissues/organs and cause more or less severe pathologies, however parasite life-cycle fails. Previous studies on schistosomula migration in experimentally infected mice revealed the majority of successfully penetrating larvae being localized in skin; the rest migrate through host body using specific migratory routes. Our recent findings showed that the proportion of worms which migrate and invade particular tissues/organs may be influenced with shift in TH1/TH2 cell immune response balance. The aim of present study was to evaluate the impact of non-specific immunostimulation (and subsequent change in polarization of systemic cell response) on schistosomula migration and development of accompanying pathologies. for this reason, naive and non-specifically immunostimulated BALB/c and C57/BL6 mice (predisposed for development of TH2 and TH1 polarized response, respectively) were infected with *T. regenti*. Host-parasite interactions (localization and abundance of schistosomula, histopathological changes in invaded tissues, systemic cell and antibody response) were followed 1–15 days post infection. Particularly, spinal cord, brain, heart, lungs, liver and kidney were searched for parasite presence; histological and immunohistochemical methods for histopathological observation were applied on sections of spinal cord and brain. Sera were tested for specific antibodies with ELISA and FACS was used for identification of specific markers for TH1 and TH2 cells in blood. The study revealed positive correlation between the shift towards TH1 response and the intensity of migration and severity of tissue injuries. The results indicate increased health risks connected with Trichobilharzia infection for mammalian hosts (potentially also human) with previously modulated cell immune response; this may occur under natural conditions e.g. due to exposure to other infectious agent.
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### O.6.11.002
**Parasite specific proliferation of chicken spleenocytes upon *in vitro* stimulation with *Eimeria tenella* antigen**

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Introduction The study aimed to establish methods for assessment of parasite specific T-cell responses in chickens infected with the coccidian *Eimeria tenella*.
Material and Methods SPF-reared female chicks were experimentally infected with approximately 1000 live sporulated *E. tenella* oocysts/bird at 18 days of age. Chicks were killed and spleens collected every second day between day 6 and 16 post infection. Cultures of spleen cells were stimulated with sonicated *E. tenella* sporozoite antigen for 96 h and proliferation was measured as incorporation of $^{3}$H-tymidine during the last 24 h of culture.
Results Using data from four experimental infections with a total of 21 chicks at each sampling day and 27 age-matched uninfected controls a clear significant increase in proliferation was observed at days 8–14 post infection compared to that observed for uninfected chicks and in control cultures without antigen. However, a large variation between birds in the magnitude of proliferative responses was observed. Moreover, high spontaneous proliferation in unstimulated cultures was also often observed. Using a three times higher infection dose less variation in parasite lesion scores was achieved compared to the standard dose. However, this higher dose did not affect the degree of interindividual variation of proliferative responses. To reduce background proliferation, depletion of $\gamma\delta$TCR expressing cells was tried. However, neither any significant decrease of background proliferation nor any significant alteration of antigen


 

specific proliferation was observed when comparing cultures with and without $\gamma\delta$TCR expressing cells.
CONCLUSION Thus, *E. tenella* specific proliferation can be detected 8–14 days post infection, but has proved difficult to assess reliably without a high number of observations. Improvements to this assay would be very valuable for studies of Eimeria immunity in the chicken.

## O.6.11.003

### *Plasmodium* sp. (lineage pCOLL4): first experimental data on virulence and development in avian hosts and mosquitoes

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Over 50 avian Plasmodium species have been described. However, the majority of the descriptions contain information mainly only about blood stages of the parasites. Deciphering life cycles of malaria parasites is time consuming and may take decades. Recent descriptions of malaria parasites include genetic information, which is crucial for barcoding and phylogenetic analysis. Recently, the lineage pCOLL4 of *Plasmodium* sp. was deposited in MalAvi database. This parasite is of broad both the distribution (South America and Europe) and specificity; it has been reported in birds belonging to seven families. However, live cycle of this parasite remains unknown. We investigated erythrocytic and exoerythrocytic development of the lineage pCOLL4 in experimentally infected domestic canaries *Serinus canaria* and its sporogonic development in three mosquito species *Culex pipiens pipiens*, *Culex pipiens* form molestus and *Aedes vexans*. A Red-Backed Shrike (*Lanius collurio*) naturally infected with the lineage pCOLL4 was caught. Eleven canaries were infected experimentally with this parasite. Additionally, mosquitoes of three species were allowed to take infected blood meals and tested for presence of sporogonic stages. This study shows that the lineage pCOLL4 is virulent and kills some canaries. Parasitemia reached 70% in some infected birds. Erythrocytic merogony is synchronized, with periodicity close to 24 h. Phanerozoites were observed in numerous organs (brain, heart, liver, lungs, spleen and muscles) of dead canaries. Numerous phanerozoites developed in the brain, indicating possible cerebral paralysis. This lineage developed numerous gametes and ookinetes, and a few oocysts in C. p. pipiens, C. p. form molestus and *A. vexans*. However, further sporogonic development is abortive; sporozoites do not develop. Because this parasite has been reported only in a few African migrants in Europe, it is likely that transmission takes place only in tropics and requires other mosquito species.

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## O.6.11.004

### Migratory pattern of *Toxocara cati* and *Toxocara canis* in pigs

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INTRODUCTION In man infections with Toxocara cati and *T. canis* may cause visceral larva migrans (VLM). The relative contribution of the two species in VLM as well as the predilection sites of *T. cati* in the human host is largely unknown. We used the pig as a model for human infection and conducted two studies to explore the migratory patterns of *T. cati* as compared to *T. canis*.
MATERIAL AND METHODS Four groups of five to seven pigs were inoculated with 50 000 (study A) or 10 000 (study B) embryonated eggs of either *T. cati* or *T. canis*. Larvae were recovered from liver, lungs, mesenterial lymph nodes, heart, brain, eyes, diaphragm, tongue and skeletal muscles at day 14 post infection (p.i.) (study A) or day 30–32 p.i. (study B) by HCl/pepsin digestion.
RESULTS A higher, but not significantly so, mean larval burden was found in lymph nodes of *T. cati* than *T. canis*, both on day 14 p.i. (134.0 vs. 99.9) and day 30–32 p.i. (3.6 vs. 1.3). The opposite trend was found in the lungs, both on day 14 p.i. (57.4 vs. 95.7; NS) and on day 30–32 p.i. (1.9 vs. 11.0; $P = 0.08$). In addition, we recovered larvae from the liver of three *T. canis* infected pigs (10, 65 and 83 larvae) on day 14 p.i. and 1 out of 7 on day 30–32 p.i. (11 larvae) whereas no larvae were found in livers of *T. cati* infected pigs. Gross pathology of livers from *T. canis* infected pigs on day 14 p.i. was more severe than *T. cati* even though numbers of white spots were similar.
CONCLUSIONS Our data suggest a different tissue distribution of *T. cati* and *T. canis* which may reflect different migratory patterns in VLM and perhaps different pathogenicity.