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Basel, Mission 21, 26th November 2009

Program and Abstracts

Diagnostics: From rapid tests to molecular diagnostics

Time	min	Title of session / presentation	Speaker
09.30-09.40	**10**	**Welcome and introduction to day**	C. Hatz C. Lengeler
09.40-10.40	**60**	**Keynote 1: Rapid diagnostic tests at the point of care** **Keynote 2: Recent advances in rapid and broad diagnostic of infectious diseases**	M. Perkins *FIND Geneva* J. Schrenzel, *Geneva*
10.40-11.00	**20**	**Tea break**	
11.00-12.30	**90**	**Parallel session 1) Diagnosis** Chairs: Hanspeter Beck and Felix Grimm 1. *Trichinella* infections in food animals: how to find them and how to prevent infections in humans – the situation in Switzerland 2. Protoscolex and metacestode antigens in the serological diagnosis of echinococcosis 3. Discovery and validation of human African trypanosomiasis staging markers 4. A qPCR assay for differential diagnosis of *Entamoeba histolytica*/*E. dispar* 5. Multiplex real-time PCR for the diagnosis of malaria: correlation with microscopy and with clinical presentation 6. Comparison of molecular methods and microscopy for detection of *Plasmodium falciparum* and *P. vivax* infections	 C.F. Frey A. Schweiger J.-C. Sanchez I. Felger G. Greub C. Köpfli
11.00-12.30	**90**	**Parallel session 2) Public health / epidemiology** Chairs: Alex Mathis and Jacob Zinsstag 1. Massive reduction of antimalarial prescriptions during programmatic implementation of Rapid Diagnostic Test in Dar es Salaam, Tanzania 2. A community-based surveillance system to assess the effects of malaria interventions 3. Molecular ex vivo diagnosis and genotyping of *Echinococcus multilocularis* 4. Emergence of *Aedes japonicus* in Central Europe 5. Le 'Centre national de référence pour les	 V. D'Acremont S. Alba B. Gottstein F. Schaffner O. Péter

		maladies transmises par les tiques' (CNRT)	
12.30-14.00	**90**	**Lunch**	
14.00-15.30	**90**	**Parallel session 3) Veterinary public health / veterinary parasitology** Chairs: Manuela Schnyder and Norbert Müller 1. Comparative assessment of fluorescence polarization and tuberculin skin testing for the diagnosis of bovine tuberculosis in Chadian cattle 2. Rabies diagnosis and cost-effectiveness of rabies control in African cities 3. A critical validation of the visual diagnosis of the beef tapeworm (*Taenia saginata*) during meat inspection 4. Clinical and laboratory findings in dogs experimentally infected with the heart worm *Angiostrongylus vasorum* and first results of a new diagnostic serological ELISA using monoclonal and polyclonal antibodies 5. Identification of an as yet unknown *Leishmania* genotype causing equine cutaneous leishmaniasis in Central Europe	 B.N. Ngandolo J. Zinsstag R. Eichenberger M. Schnyder N. Müller
14.00-15.30	**90**	**Parallel session 4) Clinical case reports** Chairs: François Chappuis and Françoise Lüthi	
15.30-15.50	**20**	**Tea break**	
15.50-16.20	**30**	**Keynote 3: Revised recommendations for the screening of toxoplasmosis**	Ch. Rudin *Basel*
16.20-16.30	**10**	**Conclusions of the day and closure**	C. Hatz C. Lengeler
16.30-17.30	**90**	**General Assembly of Swiss Society of Tropical Medicine and Parasitology (separate agenda)**	

Abstracts

1) Diagnosis

Trichinella infections in food animals: how to find them and how to prevent infections in humans – the situation in Switzerland

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Trichinella spp. have not been detected anymore in Swiss pigs or wild boar for many decades, although the parasite (*T. britovi*) was repeatedly isolated from foxes (*Vulpes vulpes*) and lynxes (*Lynx lynx*). Before 2007, routine inspection for trichinellosis was applied to pigs slaughtered in export-abattoires (concerns about 20% of the pigs) and wildlife meat for non-private consumption. The methods used were either trichinoscopy or artificial digestion. In the year 2007, Switzerland has ratified the EU Regulation 2075/2005 on the *Trichinella* inspection of susceptible animals. Since then, the number of tested animals has increased dramatically: all slaughter pigs, as well as all horses, have to be tested by artificial digestion, while trichinoscopy is no longer accepted. This presentation will focus on the possibilities and limitations of both the magnetic stirrer and automated digestion method representing those diagnostic techniques that are routinely performed by abattoires, and laboratories, in Switzerland. Furthermore, results from, and experiences with, the annual scheme for external quality assessment of these methods will be presented. Although serological detection of anti-*Trichinella*-antibodies represents an alternative diagnostic method that exhibits a high sensitivity and specificity, its suitability in routine meat inspection is controversially discussed. This presentation will introduce into the serological methods currently in use at the Swiss reference laboratory and will discuss eventual application of these methods in *Trichinella* control. Furthermore, a survey on *Trichinella* in Switzerland comparing artificial digestion with serological methods will be presented.

Protoscolex and metacestode antigens in the serological diagnosis of echinococcosis

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Diagnosis of Alveolar Echinococcosis (AE) and Cystic Echinococcosis (CE) is based upon imaging and serological techniques, as no pathognomonic clinical features exist. Currently, serological diagnosis of AE primarily relies on purified (Em2, EmG11, Em18) or recombinant antigens (recII/3-10, recEm18, recEm10). Protoscolex antigens exhibited a high diagnostic sensitivity and specificity when applied in immunoblots. In a comparative study we evaluated the diagnostic value of different antigens prepared from worms, protoscolices and metacestode material of *Echinococcus granulosus* (Eg) and *E. multilocularis* (Em) for the serological diagnosis of echinococcosis.

Sera of 56 treatment-naïve AE patients and 24 CE patients from the Echinococcosis–Clinic of the University Hospital of Zurich and of 98 Swiss blood donors were used. The antigens used were: Em2Plus™ (Bordier Affinity Products, Crissier), Eg hydatid fluid (EGHF), crude Eg protoscolices (EgP), Eg protoscolex integument (EgPI), crude Em metacestode antigen (EmC), EmG11depleted, Em protoscolex integument (EmPI), Em adult integument (EmAI). Cut-off values were evaluated by ROC (SPSS 17.0, SPSS Inc., Chicago, IL). Specifity was set at 99%.

Sensitivities for the serological diagnosis of echinococcosis were: Em2Plus: 75% (92.9% for AE); EGHF: 82.5%; EgP: 98.7%; EgPI: 94.9%; EmC: 97.5%. EmPI: 93.8%; EmAI: 92.5%.

Protoscolex and metacestode antigens of *E. granulosus* and *E. multilocularis* yield a high sensitivity for the serological diagnosis of echinococcosis at a given specifity of 99%. As these assays are not species-specific, additional tests need to be performed for species differentiation. The preparation of the antigens is easy and cheap, and the base material is widely available in endemic countries. Thus, these assays are valuable tools for epidemiological screening or serological diagnosis, particularly in underprivileged countries.

Discovery And Validation of Human African Trypanosomiasis Staging Markers

Natalia Tiberti[1], Alexandre Hainard[1], Xavier Robin[1], Veerle Lejon[2], Dieudonné Mumba Ngoyi[3], Enock Matovu[4], John Enyaru[5], Catherine Fouda[1], Joseph Mathu Ndung'u[6], Markus Mueller[7], Frédérique Lisacek[7], Alexander Scherl[1], Loïc Dayon[1], Natacha Turck[1], Jean-Charles Sanchez[1]

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Human African trypanosomiasis (HAT), also known as sleeping sickness, is a parasitic tropical disease. It progresses from a haemolymphatic first stage to a neurological second stage due to invasion of parasites into the central nervous system (CNS). As treatment depends on the stage of the disease, there is a critical need for tools that efficiently discriminate the two stages of HAT. We hypothesized that markers of brain damage and inflammation-related proteins could individually or in combination indicate the CNS invasion by the parasite.

Cerebrospinal fluid (CSF) originated from untreated, parasitologically confirmed *Trypanosoma brucei gambiense* patients. They were staged on the basis of CSF white blood cell (WBC) count and presence of parasites in CSF. Discovery and verification experiments were performed using either proteomics or classical immunoassays (ELISA and mBSA) on a cohort of 21 stage 1 and 79 stage 2 HAT patients. Panel selection was performed by optimized threshold based calculations (RIL) using all available markers.

Pre-validation experiments performed on 16 out of the 50 discovered potential staging markers showed that CXCL10 most accurately ($p<0.0001$, Mann-Whitney U test) distinguished stage 1 and stage 2 patients, with a sensitivity of 84% and specificity of 100%. CXCL10 was also clearly associated to the severity of neurological signs ($p<0.0001$, Kruskal-Wallis). RIL analysis defined a panel comprising CXCL10, CXCL8 and H-FABP that improved the detection of stage 2 patients to 97% sensitivity and 100% specificity.

This study highlighted the value of CXCL10 as a single biomarker for staging *T. b. gambiense*-infected HAT patients. Its further combination with H-FABP and CXCL8 resulted in a panel, which efficiently rules-in stage 2 HAT patients. The health care impact of this potential discovery on HAT, a neglected African disease that only affects the rural poor, is critical to allow more appropriate therapeutic interventions.

(1) A. Hainard et al, A combined CXCL10, CXCL8 and H-FABP panel for the staging of human African trypanosomiasis patients; PLoS Neglected Tropical Diseases 2029 (In Press).

A qPCR assay for differential diagnosis of *Entamoeba histolytica* / *E. dispar*

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A new quantitative Real-Time PCR assay was developed for differentiation between *Entamoeba histolytica* and *Entamoeba dispar*. For a duplex qPCR assay we have designed two species-specific probes from the *E. histolytica* and *E. dispar* 18S rRNA genes which are present in 100-200 copies per genome. Objectives were to optimize an existing assay previously used in routine diagnostics and to compare sensitivity of both assays.
Sensitivity of qPCR was determined by using dilutions of cloned plasmid control. Sensitivity was 5 plasmids per reaction corresponding to 0.05 genomes (based on 100 gene copies/genome). Specificity was tested with DNAs from stool samples infected with microscopically confirmed pathogenic, non-pathogenic and facultative pathogenic organisms present in stool samples.
We have focused on microscopy-positive but PCR-negative stool samples. When using the originally extracted DNA, stored at -20°C, 6/14 previously negative sample were positive by qPCR. All showed high Ct values indicating low parasite densities at around the detection limit of standard PCR. All positive results by standard PCR were confirmed by qPCR.
qPCR was found superior to standard PCR with respect to greater sensitivity and much reduced handling time. The adoption of standard cycling conditions lead the way to parallel processing of multiple qPCR tests in a single Real-Time run.

Multiplex real-time PCR for the diagnosis of malaria: correlation with microscopy and with clinical presentation.

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Malaria is generally diagnosed by microscopy and rapid antigen testing. Molecular methods have been recently developed. We thus investigated the contribution of a quantitative multiplex PCR for malaria diagnosis assessing i) the agreement between PCR-based identification and microscopy, ii) the correlation between the parasite load determined by this quantitative PCR and by microscopy and iii) the correlation of parasite load with clinical severity.
For 83 patients positive by microscopy, the first EDTA-blood sample was tested by PCR to confirm smear-based species identification. Number of parasites/mL of blood was assessed daily using both microscopy and PCR. Clinical criteria of severity were retrieved from medical files and were compared to microscopy and PCR quantification.
Among the 83 patients tested, 1 was positive by microscopy only and 82 were positive by microscopy and PCR. Nine identifications were discordant. All these 9 discordant results concerned co-infections with 2 or 3 species and were attributed to inaccurate phenotypic identification of mixed cases. The number of parasites/mL of blood generally decreased rapidly after treatment start with similar decay curves, obtained with both microscopy and PCR. Clinical severity was significantly correlated with high parasite load as determined by microscopy ($p=0.031$) and PCR ($p=0.0297$).
Our PCR proved especially useful to identify mixed infections. The quantification obtained by PCR closely correlated with microscopy-based quantification and with disease severity.

Comparison of molecular methods and microscopy for detection of *Plasmodium falciparum* and *P. vivax* infections

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Plasmodium falciparum and *P. vivax* together account for the majority of human malaria cases. For sustained control or eradication of malaria, basic epidemiological knowledge on the infection dynamics and species interactions is essential. Precise estimates of parasite prevalence, including microscopically subpatent infections, can be achieved by PCR-based detection methods. We have followed up a cohort of 268 children aged 1 to 5 years in a region of Papua New Guinea with sympatric *P. falciparum* and *P. vivax*, occurring at a prevalence by PCR of 49.6% and 53.0%, respectively at baseline. Blood samples were taken regularly and analyzed by light microscopy and post-PCR ligase detection reaction (LDR) for infection with different *Plasmodium* species.

Over the entire study time, in 1390 out of 4877 (28.5%) blood slides *P. falciparum* was diagnosed by microscopy. By LDR an additional 833 *P. falciparum* positive samples were observed, leading to a total of 2223 out of 4877 (45.5%) *P. falciparum* positive samples. In 2186 samples (44.8%) *P. vivax* was diagnosed by microscopy. LDR revealed an additional 766 *P. vivax* positive samples, leading to a total of 2952 out of 4877 samples (60.5%).

Positive samples were further analyzed by PCR amplification of size polymorphic *P. falciparum* and *P. vivax* markers in order to determine multiplicity of infection and to track individual clones longitudinally.

177 samples were positive for *P. vivax* by microscopy but not LDR. 114 (64%) of them were positive by at least one genotyping marker. 63 samples were microscopy negative but LDR positive for *P. falciparum*, 23 of them were *P. falciparum* positive by the genotyping marker msp2. Thus the nested PCR of polymorphic marker genes (1 marker for *P. falciparum* and 2 markers for *P. vivax*) was more sensitive than LDR.

Multiplicity of infection (MOI) was higher in *P. vivax* than of *P. falciparum* with a mean of 2.9 concurrrent *P. vivax* infections per positive carrier versus 1.5 concurrent *P. falciparum* clones per carrier. High MOI in one species did not affect MOI of the other species, whereas overall, mixed species infections were less prevalent than expected. This could reflect heterogeneity in exposure to Malaria within the study area: the observed high MOI of each species in mixed species infections would indicate a history of high exposure of certain individuals possibly related to geographic or environmental factors.

2) Public health and epidemiology

Massive reduction of antimalarial prescriptions during programmatic implementation of Rapid Diagnostic Test in Dar es Salaam, Tanzania

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Presumptive treatment of all febrile children with antimalarials leads to massive over-treatment and a huge wastage of drugs, especially in areas with moderate or low endemicity. Laboratory confirmed diagnosis would drastically reduce antimalarial consumption, provided the test result is taken into account by the attending clinicians. We aimed to assess the effect of implementing malaria rapid diagnostic tests (RDTs) as first-line diagnostic tool in routine management of febrile patients living in a moderately endemic area on the prescription of first line antimalarials.

RDTs were introduced after training of all health workers of 3 district hospitals, 3 health centers and 3 dispensaries. Three similar health facilities without RDT implementation were selected randomly as controls. Supervision, problem-solving and quality control of RDT performance took place every 3 months. Data on antimalarial use during a period of 15 months before and 18 months after RDT initiation were compiled from ledger books of storage places in each health facility.

When comparing consumption of ALu during 3 months prior to RDT implementation with 18 months post-initiation, there was a mean of 6-fold (range: 2 - 26) decrease in intervention facilities and 1.7-fold decrease in control health facilities. When comparing consumption of quinine vials during 15 months pre- with eighteen months post-initiation, there was a mean of 3-fold (range:2 - 6) decrease in intervention facilities and no decrease in control health facilities.

The overall proportion of febrile patients who were prescribed antimalarials decreased from 82% to 24%. For non-febrile patients, antimalarial prescriptions decreased from 37% to 5%. In total, for 100 patients attending with medical problems, 57 antimalarial treatments could thus be saved.

Programmatic implementation of RDTs in a moderately endemic area where microscopy is available reduced drastically over-treatment with antimalarials. Properly trained clinicians with adequate support complied with the recommendation of not treating patients with negative results. RDTs used as first-line diagnostic tool have a huge potential for reducing inappropriate prescriptions and hence improve management of patients.

A community-based surveillance system to asses the effects of malaria interventions

Sandra Alba, Manuel Hetzel, Angel Dillip, Iddy Mayumana, Christian Lengeler, Mathew Alexander, Rose Nathan, Brigit Obrist, Alexander Schulze, Flora Kessy, Hassan Mshinda

The ACCESS Programme aims at understanding and improving access to prompt and effective malaria treatment and care in a rural Tanzanian setting with a set of integrated interventions.
To evaluate the programme's impact on reported incidence of fever and severe malaria disease at both the community and health facility levels, and to investigate the value of community-based reporting for routine malaria control programme monitoring.
This work was implemented within the Ifakara Demographic Surveillance System (DSS) which comprises a total population of 80,000 in southern Tanzania. Besides data on mortality, the DSS staff routinely collected data on reported incidence of fever (2 week recall) and severe malaria disease in the community. In parallel we collected fever data from the 15 health facilities in the area.
Reported fever rates in the community decreased from 47.2 to 41.4/1000 person weeks (IRR=0.94, $p<0.001$) between 2005 and 2007. The rates fever in the health facilities decreased slightly from 19.9 to 18.8/1000 person-weeks between 2005 and 2007 (IRR=0.96, $p<0.001$). A good temporal and quantitative relationship was found between community-reported fever and health facility malaria diagnoses, suggesting that the former could be used for routine monitoring. Moreover, good internal and external consistency was found with a separate treatment seeking survey in the same community and with national data.
The trends of fever cases indicated a reduction in malaria risk. This conclusion is strengthened by the great consistency of the data collected from four independent sources.

Molecular ex vivo diagnosis and genotyping of *Echinococcus multilocularis*

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The primary diagnostic identification of parasite materials in biological specimens (resections or biopsies), and also the assessment of the viability of parasite samples following chemotherapy or other treatment can be of significant prognostic value for human AE patients. Furthermore, spatially related genotyping of detected parasites may help to understand the epidemiology of the disease. However, *Echinococcus multilocularis* isolates, so far, exhibited no significant genetic differences using classical nuclear and mitochondrial targets (Haag et al., 1997). Thus, there was need for new more sensitive genetic markers, such as appropriate microsatellite targets.
Conventional diagnostic PCR (Georges et al., 2004); real-time RT-PCR (Matsumoto et al., 2006); EmsB-microsatellite-PCR for genotyping (Knapp et al., 2007).
Laboratory application of conventional diagnostic PCR proved useful for complicated cases of extrahepatic AE, e.g. pancreatic AE (Diebold-Berger et al., 1997); however, a negative result on a thin needle aspiration sample does not rule out disease. Determination of parasite viability, especially after long-term medical treatment, has also been achieved by real-time RT-PCR in the experimental murine model so far (Matsumoto et al., 2006), the method appears suitable for assessing human AE cases.
Genetic analyses of *E. multilocularis* in foxes showed that the northern Alpine arch harbored the highest genetic richness and diversity, as compared to surrounding areas in northern and eastern Europe. Presently, cases of human AE are investigated in view to determine the impact of the genetic polymorphism to the infection pattern within the affected human populations.

Diebold Berger S, Khan H, Gottstein B, Puget E, Frossard JL, Remadi S: Cytologic diagnosis of isolated pancreatic alveolar hydatid disease with immunologic and PCR analyses - A case report. Acta Cytologica 41:1381-1386 (1997).

Georges S, Villard O, Filisetti D, Mathis A, Marcellin L, Hansmann Y, Candolfi E: Usefulness of PCR analysis for diagnosis of alveolar echinococcosis with unusual localizations: two case studies. J Clin Microbiol. 42:5954-5956 (2004).

Haag KL, Zaha A, Araújo AM, Gottstein B: Reduced genetic variability within coding and non-coding regions of the *Echinococcus multilocularis* genome. Parasitology 115:521-529 (1997).

Knapp J, Bart JM, Giraudoux P, Glowatzki ML, Breyer I, Raoul F, Deplazes P, Duscher G, Martinek K, Dubinsky P, Guislain MH, Cliquet F, Romig T, Malczewski A, Gottstein B, Piarroux R: Genetic diversity of the cestode *Echinococcus multilocularis* in red foxes at a continental scale in Europe. Plos Negl Trop Dis 3(6): e452 (2009).

Knapp J, J.M. Bart, M.L. Glowatzki, A. Ito, S. Gerard, S. Maillard, R. Piarroux, B. Gottstein: Assessment of microsatellite polymorphism for improving the spatial distribution tracking of *Echinococcus multilocularis.* J Clinical Microbiology, 45: 2943-2950 (2007).

Matsumoto J, Müller N, Hemphill A, Oku Y, Kamiya M, Gottstein B: 14-3-3- and II/3-10-gene expression as molecular markers to address viability and growth activity of *Echinococcus multilocularis* metacestode. Parasitology 132: 83-94 (2006).

Emergence of *Aedes japonicus* in Central Europe

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A damaged mosquito specimen resembling *Ae. albopictus*, the Asian tiger mosquito, collected in the canton Aargau and sent to our laboratory* in July 2008, was identified as *Ae. japonicus*, which was not known to occur in Switzerland. Field investigations were implemented in order to determine the distribution and the spread of the species in 2008-09.
Larval collections in potential breeding sites focused on small man-made containers like vases in cemeteries. In 2008, the surveyed area was gradually extended from positive sites in all directions until a crown of negative sites surrounding the positive sites was determined. In 2009, municipalities along five transects outgoing from the known colonized area were investigated.
In 2008, *Ae. japonicus* was detected in 160 containers, mainly in vases (73.8%), in Switzerland (36 municipalities) and neighbouring Germany (2 municipalities) (Schaffner *et al.* 2009). The colonized area covered approximately 1,400 km^2. At sites where *Ae. japonicus* was present, it occurred more frequently than indigenous species In 2009, the species was found in 9 new locations revealing a spread in all directions.
This is the first finding of proliferation and spread of an invasive mosquito in Central Europe. *Ae. japonicus* is known as an invasive species (ISGG, 2009) and as a competent laboratory vector of several arboviruses (Williges *et al*. 2008). Further studies should monitor the rapidity of its spread as well as determine the bionomics of this species, in order to assess its vector potential for native and exotic pathogens in the local environment. This is particularly of relevance as *Ae. japonicus* is breeding in urbanized environments. Invasive as well as vector potential render this species a potential threat for animal and human health, and justify the implementation of preventive surveillance and control measures.

* Reference laboratory for arachno-entomology for the Swiss federal veterinary office.

ISSG (Invasive Species Specialist Group). (2009) Global Invasive Species Database. Available from: http://www.issg.org/database/welcome/. [Accessed 03 September 2009].

Williges, E., Farajollahi, A., Scott, J.J., McCuiston, L.J., Crans, W.C. & Gaugler, R. (2008) Laboratory colonization of *Aedes japonicus japonicus*. Journal of the American Mosquito Control Association, 24, 591-593.

Schaffner F., Kaufmann C., Hegglin, D. & Mathis A. (2009) The invasive mosquito *Aedes japonicus* in Central Europe. Medical and Veterinary Entomology, in press.

3) Veterinary public health / veterinary parasitology

Comparative assessment of fluorescence polarization and tuberculin skin testing for the diagnosis of bovine tuberculosis in Chadian cattle

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Effective surveillance of bovine tuberculosis (BTB) in developing countries where reliable data on disease prevalence is scarce or absent is a precondition for considering potential control options. We conducted a slaughterhouse survey to assess for the first time the burden of BTB in Southern Chad. Altogether, 954 slaughter animals were consecutively sampled and tested using the single intra-dermal comparative cervical tuberculin (SICCT) test, a recently developed fluorescence polarization assay (FPA) and routine abattoir meat inspection after slaughter. Gross visible lesions were detected in 11.3% (CI: 9.4–13.5%) of the animals examined and they were mostly located in the lymph nodes and the lung. Significantly more Mbororo zebus (15.0%) were affected by lesions than Arab zebus (9.9%; OR = 2.20, CI: 1.41–3.41%; $p < 0.001$). Of all animals tested, 7.7% (CI: 6.2–9.6%) reacted positively to SICCT if OIE guidelines were applied. However, receiver operating characteristic (ROC) analysis using Mycobacterium tuberculosis complex (MTBC) infected animals as the positive population and lesion negative animals as the negative population, revealed a better SICCT performance if the cut-off value was decreased to >2 mm. SICCT reactor prevalence rose to 15.5% (CI: 13.3–18.0%) and FPA did not perform better than SICCT, when this setting adapted cut-off was applied. Animal health policy implications are discussed.

Rabies diagnosis and cost-effectiveness of rabies control in African cities

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Human rabies in developing countries can be prevented through interventions directed at dogs. Potential cost-savings for the public health sector of interventions aimed at animal host reservoirs should be assessed. On request of the Chadian partners standard immunofluorescence rabies and the new direct rapid immunohistochemial test (dRIT) (see contribution S. Dürr) were established in N'Djaména, Chad. For every suspected dog, a questionnaire was filled for the number of human exposures were assessed weekly, during six years. A cohort of more than 300 dogs was followed to assess dog reproduction and mortality rates. Small scale intervention trials showed the feasibility and sufficiently high coverage (>70%) of parenteral dog mass vaccination when vaccination campaigns were free. However coverage was reduced to 20% when dog owners had to pay for dog vaccination. Available deterministic models of rabies transmission between dogs were extended to include dog to human rabies transmission. Model parameters were fitted to routine weekly rabid dog and exposed human cases. We simulated the effects of mass dog vaccination and the culling of a percentage of the dog population on human rabies incidence. A single parenteral dog rabies mass vaccination campaign achieving a coverage of least 70% appears to be sufficient to interrupt transmission of rabies to humans for at least six years. The cost-effectiveness of mass dog vaccination was compared to post-exposure prophylaxis (PEP), which is the current practice in Chad. PEP does not reduce future human exposure. Its cost-effectiveness is estimated at US$ 46 per DALY averted. Cost-effectiveness for PEP together with a dog vaccination campaign, breaks even with cost-effectiveness of PEP alone after almost 5 years. Beyond a time frame of 7 years, it appears to be more cost-effective to combine parenteral dog vaccination campaigns with human PEP compared to human PEP alone.

A critical validation of the visual diagnosis of the beef tapeworm (*Taenia saginata*) during meat inspection

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Taenia saginata is the common tapeworm in humans (as final host) in our region; cattle act as the only intermediate hosts. Although this parasite causes low morbidity, *T. saginata* cysticercosis in cattle generates financial losses and represents a still unsolved problem with respect to food safety. The Swiss legal routine meat inspection (according to EU legislation) for the diagnosis of *T. saginata* cysticercosis in cattle includes cutting musculature in the inner and exterior cheek muscles and slicing at least two times the heart longitudinally, followed by visual examination for the presence of parasitic cysts. Several studies estimate the sensitivity of this procedure to be around 30%. An ongoing study at our Institute aims at increasing the sensitivity for *T. saginata* cysticercosis at meat inspection.

Several additional cuts at hearts from a total of 1088 slaughtered cattle in three EU-approved abattoirs in Switzerland were made. Cysts were classified by the usual organoleptic methods during meat inspection and by microscopical and molecular analyses in our laboratory.

The standard procedure identified 1.8% of the animals (20/1088) harboring cystic lesions. With the additional investigation more than twice as many infected animals were detected, yielding an apparent prevalence of the investigated group of 4.4% (48/1088).

Increasing the diagnostic sensitivity at meat inspection is an important point in a comprehensive program in combating bovine cysticercosis. Serological tests with serum and meat-juice samples have been developed, and will be validated for the epidemiological situation in Switzerland.

Clinical and laboratory findings in dogs experimentally infected with the heart worm *Angiostrongylus vasorum* and first results of a new diagnostic serological ELISA using monoclonal and polyclonal antibodies

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Angiostrongylus vasorum, a parasite living in the right heart and pulmonary arteries, is increasingly reported Europe-wide in dogs and foxes. Infected dogs may present severe respiratory symptoms, but also haematological and neurological signs have been described. The goal of the present study was to follow up the development of clinical signs and haematological changes, to test the sensitivity of larval detection in faeces and to document the pathological changes in an experimental setting. Infections with 50-500 *A. vasorum* third stage larvae from experimentally infected snails were induced in 3 experiments through oral or intragastral administration involving 22 dogs (Beagles) in total. Dogs were examined weekly clinically and daily coproscopically by the funnel migration technique. *Post-mortem* examinations (16 dogs) included a reverse lung perfusion technique and necropsy in order to isolate adult *A. vasorum*. Increased respiration rates and respiratory sounds were observed starting from day 42 post infection (dpi). Typical observations, increasing with time after infection, were panting, abdominally accentuated and deepened respiration with intensified inspiratory and/or expiratory sounds. Faeces containing blood and mucus were occasionally observed. Loss of appetite with weight reduction was common. Dogs of trial 1 developed neutrophilic leucocytosis with left shift, particularly from 49 dpi on, as well as occasional mild anaemia, thrombocytopenia, basophilia, eosinophilia and monocytosis. Coagulation parameters (PT, PTT, TT) were constantly within reference ranges. L1 were detected 47-55 dpi in all dogs. Patency lasted until the end of the study (56-90 dpi). During patency, faecal examinations were not consistently positive. Upon necropsy, 10-170 worms were recovered. The lungs of all dogs showed large confluent areas that were hardened, raised and discoloured, with frequent haemorrhagic patches. Pneumonia, thrombi and parasites were confirmed by histology. These studies show that infections starting from 50 infectious L3 of *A. vasorum* have a massive impact on lung tissues and therefore on the health of affected dogs, particularly after prepatency, although only mild laboratory abnormalities are evident. Serology for circulating parasite antigens revealed first positive results around the beginning of patency and persisted positive until the end of the experiment.

Identification of an as yet unknown *Leishmania* genotype causing equine cutaneous leishmaniasis in Central Europe

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The species *Leishmania infantum* has been reported as causative agent of cutaneous leishmaniasis in horses that had been sporadically recorded as suspected autochthonous cases in various European countries including Spain, Portugal, and Germany. The clinical symptoms in these animals were similar to those related to American equine leishmaniasis caused by New World species such as *Leishmania braziliensis*. Now, we present clinical, histological, and initial molecular epidemiological findings related to nine new cases of equine leishmaniasis in Germany and Switzerland.
The diagnosis in the horses was based on clinical (presence of skin nodules, cutaneous lesions), and histological (presence of cutaneous infiltrates composed of possibly parasitized macrophages, as well as small numbers of lymphocytes, multinucleated giant cells and neutrophils) findings. Furthermore, the molecular identification of *Leishmania* sp. in skin biopsy samples was based on an approach including a diagnostic PCR targeted to the internal transcribed spacer 1 (ITS1) of ssrRNA.
We identified a novel etiological agent of cutaneous leishmaniasis in horses that, at least for some cases, sporadically appeared as autochthonous infections in geographically distant regions of Germany and Switzerland. Comparative sequence analysis of diagnostic ITS1-PCR products classified the respective isolates as neither Old World nor New World *Leishmania* species. However, these isolates exhibited a close phylogenetic relationship to *Leishmania* sp. *siamensis*, an organism recently identified in a visceral leishmaniasis patient from Thailand.
The present findings suggest the existence of a novel *Leishmania* species in Central Europe that causes cutaneous leishmaniasis in horses. Future investigations will have answer the questions whether the present form of leishmaniasis remains a sporadic and local phenomenon in Central Europe or whether it has to be considered as an emerging, and perhaps zoonotic, disease with a potential to spread over the European continent and other parts of the world.