ABSTRACT O_Ple1_1

Background and Aim: From the 19th century to now, echinococcosis/hydatid disease has raised interest in various scientifically leading countries, related to the main endemic areas of the disease. Greek- and Latin-derived languages, as well as German and Russian, and more recently Chinese, have contributed to establish the vocabulary of the field. Translations from one language to the others as well as from these languages to English are sometimes source of ‘false-synonyms’ that may lead to misunderstanding. Because of the worldwide distribution of the disease, and because multidisciplinary and multicenter studies have become the rule to study echinococcosis in humans/animals/environment, a common and fixed language in English has become crucial. The World Association of Echinococcosis is legitimate to provide worldwide accepted terms and expressions that should be used in international publications and communications at conferences and meetings. The aim of the work we intend to achieve is 1) to provide a basis for discussion and decision on the accepted words/expressions in English to designate the parasites of the *Echinococcus* genus, and the related diseases; 2) to reach a consensus after further scientifically- and/or historically-based discussion with the recognized specialists of each field; 3) to publish this consensus in an internationally indexed journal.

Method: At the 27th World Congress of Echinococcosis (Oct 4-7, 2017): we will list and present various synonyms used in publications both in the human and veterinary medical field and in related disciplines (parasitology, pathology, taxonomy, genotyping, immunology etc.), and we will give some examples of ‘sensitive issues’ regarding words and expression commonly used in the scientific/medical literature on echinococcosis, with the ‘pros’ and ‘cons’ for their usage (based on etymology, history, biology, decision of international societies…), in 3 domains: *Species nomenclature; Parasite biology/immunology; and Clinical aspects.* We also propose to constitute Working Groups on each of the domains, on a voluntary basis. From Oct 7, 2017 to Dec 31, 2017: there will be email exchanges between the participants in the 3 working groups on the various words/expressions to enrich the scientific/terminological basis for further decisions, and a ‘writing committee’ will be designated to prepare the successive drafts of a position paper. The international journal “Parasite” has accepted to waive publication fees for this paper.

Expected Results: In Jan 2018: we expect final decisions on the definitively accepted words/expressions. From Feb 1st, 2018 to Jun 30th, 2018: the manuscript will be prepared for
publication of the ‘WAE nomenclature on *Echinococcus* spp/Echinococcosis’. Submission of the paper is expected in July 2018, and publication within the year 2018.

*Legend for author identification:

Family name and first name: in CAPITAL LETTERS

First name: *italics*

Presenting author: **bold and underlined**

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<table>
<thead>
<tr>
<th>Oral</th>
<th>X</th>
<th>Session</th>
<th>Ple2</th>
<th>Video</th>
<th>Poster</th>
</tr>
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</table>

**Title**
Integration of animal health and public health surveillance sources to exhaustively inform the risk of echinococcosis in Rio Negro, Argentina

**Authors**
MARCOS A AREZO, RAYMOND BOAZ, ANA CORBERAN, ANDREW LAWSON, EDMUNDO LARRIEU, VICTOR J DEL RIO VILAS

**Affiliation**
Public Health of Rio Negro, Argentina; University of Surrey, UK

**ABSTRACT O_Ple2_1**

**Aim:** To explore the application of analytical models to aggregate the evidence from all the surveillance sources and develop a better understanding of the epidemiology of echinococcosis in Rio Negro.

**Methods:** Cystic echinococcosis is a parasitic zoonosis endemic in large swathes of South America. In Rio Negro Province, Argentina, the consistent implementation of control measures since 1980 has led to a significant reduction of the disease incidence. Throughout this >30 year period, the echinococcosis programme has conducted a number of surveillance activities aimed to monitor the impact of their control efforts. In addition to the clinical cases detected via passive surveillance, active surveillance in the form of ultrasound screening of all 7 to 14 years old children attending school was implemented. Surveys of sheep farms to monitor environmental exposure, especially targeting lambs and resident dogs to monitor recent infection, and adult sheep to assess earlier infection, were also conducted, albeit not in a regular fashion due to logistics and budgetary constraints. With this information, we apply spatial-temporal Bayesian Hierarchical models (BHM) to, separately first and then jointly, analyze the animal and public health data for the period 2003 to 2016, and for 18 health areas in Rio Negro. We compute joint parameters to inform the shared spatial animal and human case distribution, as well as the posterior estimates of the exceedance probability of risk (Lawson 2013).

**Results:** The incorporation of spatially structured random effects and time dependence in our models smoothed the crude animal risks across the 18 health areas, and returned posterior estimates of the probability of recent infection (by only considering cases in lambs and resident dogs). Joint models of children screening data and passive cases showed the best fit among an array of alternative parameterizations and indicate that the two surveillance sources capture similar spatial and temporal risk patterns. The southwest region of the province shows the highest risk with exceedance probabilities >0.8. Across all 18 health areas the risk appears to decline throughout the study period. In some cases joint models of animal and human cases returned a better fit than human-only models but the estimated parameters pertaining to animal counts were not significant in these models. Hence this suggests that animal incidence is only weakly related to human incidence. However, the incorporation of a lagged-time term modulating the effect of animal cases on human risk, up to 6
years after the occurrence of animal cases, did not significantly improve our results, and a simpler human-only model achieves an enhanced fit when fitted year-by-year.

**Conclusion:** Cases in dogs and lambs, together with the findings from the screening of children indicate recent transmission. This would support the ongoing screening programme. The spatial concurrence of active and passive human cases might reflect the later implementation of the former following positive findings by the latter. Discrepancies between surveillance sources should be investigated. Our results will inform such investigations and refine sampling schemes with updated design prevalence estimates derived from the joint models.

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**Title:** Are the vegetables sold in Tunisian markets contaminated by *Echinococcus granulosus* eggs?

**Authors:** M’RAD SELIM, OUDNI-M’RAD MYRIAM, CHAÂBANE-BANAOUES RAJA, MEZHOUD HABIB, BABBA HAMOUUDA

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**ABSTRACT O_Ple2_2**

**Background and Aim:** With an annual surgical incidence (SI) averaging 12.6/100000 inhabitants, Tunisia is one of the most endemic areas amongst the Mediterranean countries. Humans may become infected by ingesting embryonated eggs through contaminated vegetables/water or by direct contact with dogs. In Tunisia no information on the fresh vegetables contamination by *E. granulosus* eggs is currently available and their roles in human contamination are undetermined. The aim of this work is to evaluate the contamination rate of vegetables with *E. granulosus* eggs and their putative role in the transmission of hydatidosis to humans.

**Material and methods:** Fresh vegetables marketed in six Tunisian regions were collected: Monastir and Tozeur (hypo endemic areas), Sousse, Mahdia and Gafsa (mesoendemic regions) and Kasserine (hyperendemic area). The 245 unwashed samples composed of Swiss chard (*Beta vulgaris*), celery (*Apium graveolens*), spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*), mint (*Mentha spicata*), parsley (*Petroselinum sativum*) and green onions (*Allium fistulosum*) were purchased from major vegetable markets. The parasites eggs and oocysts were recovered using a sucrose flotation. Morphological and molecular identifications were used to identify helminth eggs and protozoan cysts.

**Results:** Helminth eggs were found in 14.10% of the vegetables but only three samples (1.22%) were contaminated by *E. granulosus* eggs. No correlation between the vegetable contamination and the human SI was observed. Thus, the samples contaminated by *E. granulosus* eggs provided from Monastir (hypoendemic) markets whereas the vegetables from Kasserine (Hyperendemic) region were free of eggs.

**Conclusion:** Considering the low positive samples observed, the human contamination in Tunisia doesn’t seem to be linked to the consummation of raw vegetables. The high prevalence in humans is strongly related to human behavior and hygiene.

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Title | Oral | Session | Ple2 | Video | Poster
Title | Interlinks between wildlife and domestic cycles of *Echinococcus* spp in Kenya

Authors | DOROTHY KAGENDO, JAPHET MAGAMBO, ERIC MUCHIRI, EBERHARD ZEYHLE, ERASTUS MULINGE, CECILIA MBAE, MARION WASSERMANN, PETER KERN, THOMAS ROMIG

Affiliation | "Meru University of Science and Technology, Meru, Kenya
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ABSTRACT O_Ple2_3

**Background and Aim:** The aim of the study was to determine possible interactions between wildlife and domestic cycles of *Echinococcus* species from Maasai Mara and Samburu wildlife-domestic interfaces in Kenya.

**Methods:** To investigate possible inter-links between sylvatic and domestic cycles of *Echinococcus* spp., 729 faecal samples from wild carnivores (Samburu 342 and Maasai Mara 387) and 406 from domestic dogs (Samburu) were analyzed. Amplification of a 200 bp fragment of the mitochondrial NAD1 gene was done on single eggs from the 74 samples that contained taeniid eggs. The amplicons were sequenced, edited and analyzed with GENtle VI.94 program. Sequences obtained were compared with those in Genbank using BLAST. The environmentally collected faecal samples were subjected to molecular identification of host species origin.

**Results:** Out of the 53 positive wild carnivore samples (Samburu 27 and Maasai Mara 26), 521 taeniid eggs were isolated, of which 183 yielded amplicons. In Samburu they belonged to *E. granulosus* s.s., (31), *E. felidis* (53) *E. canadensis* G6/7 (2), *Taenia hydatigena* (9), *T. multiceps* (1), and *T. saginata* (1), while in Maasai Mara *E. granulosus* G1-3 (1), *E. felidis* (57), *T. multiceps* (6), and *T. hydatigena* (22) were found.

Similarly, molecular analysis was done with 304 eggs isolated from 21 domestic dog faecal samples from Samburu, 92 of which yielded amplicons. Species found were *E. granulosus* s.s. (9), *E. felidis* (47), *T. multiceps* (7), *T. hydatigena* (10), *T. madoquae* (10), and 9 undetermined *Taenia* spp.

Molecular host species identification of 26 faecal samples from Maasai Mara showed 4 lions, 17 spotted hyenas and one wild dog, host origin of four samples could not be verified. The 27 faecal samples from Samburu included 3 lion, 13 spotted hyena and 6 wild dogs. Five samples yielded no amplicons with the range of host primers used. Distribution of *Echinococcus* and *Taenia* spp. varied with the host species, the highest diversity was found in hyena samples. Most animals had mixed infections of *Echinococcus*, *T. multiceps* and *T. hydatigena*.

**Conclusion:** The results show a clear overlap of the various taeniids between wild carnivores and domestic dogs. However, data from intermediate hosts are still required to get insights into the lifecycles. Also, as the parasite identification rests on eggs in faeces, possible coprophagy of some carnivores (e.g. hyenas and domestic dogs) is certainly a source of error, as exemplified by the presence of *T. saginata* in hyena faeces.
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<table>
<thead>
<tr>
<th>ORAL</th>
<th>X</th>
<th>Session</th>
<th>Ple2</th>
<th>VIDEO</th>
<th>POSTER</th>
</tr>
</thead>
</table>

### Title
It’s a small world for tiny worms: the European strain of *Echinococcus multilocularis* in the new world

### Authors
MASSOLO ALESSANDRO, C. KLEIN, D. GRANT, JENNY KNAPP, A.M. SANTA, S. BELGA, K. DOUCETTE, J. PREIKSAITIS, KOWALEWSKA-GROCHOWSKA, BANU SIS, STAN HOUSTON

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### Background and aim:
Local human infections with *Echinococcus multilocularis* (*Em*), the causative agent of Alveolar Echinococcosis (AE), were reported only twice in continental North America (NA). Since 2012, research on wild and domestic canids in urban areas in Alberta (AB), Canada, indicated regular presence of *Em* in coyotes, raising concerns for transmission to humans. Since then, four new human cases were reported in AB. We aimed to characterize the parasite strain from wild and domestic animals to understand the origin of these infections, and to collect eco-epidemiological data on these patients to study the emergence of this disease in NA.

### Methods:
We collected *Em* specimens from coyotes from AB, British Columbia and Manitoba, and from foxes, domestic dogs, and small mammals from AB. We also acquired bioptic liver tissue from all the human patients. Following DNA extraction, we carried PCR analyses using end-point (PCR), real-time (qPCR) and droplet digital PCR (ddPCR) on 3 mitochondrial DNA (MtDNA) loci, and submitted for Sanger sequencing. Resulting sequencing data were used to identify single nucleotide polymorphisms (SNPs) and to strain-type each specimen into known strains found in NA and Europe (EU). Moreover, we conducted fragment analysis on a microsatellite sequence (*EmSB*) currently used to characterise the genetic variability of *Em* to estimate similarity between the strains found in AB, BC and MB and the ones in EU. *EmSB* data were used first calculating a non-parametric distance (Bray-Curtis distance) between different specimens, and then using a hierarchical clustering approach to identify similar haplotypes.

### Results:
Our data indicated that 1) a strain very similar to the European strain is the one commonly found in both wild and domestic animals; 2) the variability in *EmSB* in Western CA is very little when compared to the one observed in EU suggesting a relatively recent invasion; 3) the strains in Western Canada present SNPs that seem to be typical of the endemic strains and different from the original EU strain; 4) the travel history of all patients seems to support a local infection; 5) the parasitic DNA extracted from 2 patients showed the SNPs typical of Western CA.

### Conclusion:
Our results strongly suggest that the EU strain found in Western CA is likely due to a recent invasion and this might have changed the infection risk for people, being the EU strain known to be highly pathogenic, and so we may expect more AE cases across NA in the near future.

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FREE COMMUNICATIONS  | Oral  | X  | Session  | Ple2  | Video  | Poster

Title  | 27 year monitoring of an alveolar echinococcosis hotspot in south-Gansu

Authors  | PATRICK GIRAUDOUX, EVE AFONSO, JENNY KNAPP, WANGZHONG JIA, HONGBIN YAN, DAZHONG SHI, YUMIN ZHAO, ANNE-CLAUDE GOYDADIN, DOMINIQUE RIEFFEL, TAO JING, DOMINIQUE A VUITTON, PHILIP S CRAIG

Presenting author affiliation  | WHO-Collaborating Centre for Prevention and Treatment of Human Echinococcosis; Chrono-environment lab, University of Bourgogne Franche-Comté & Institut Universitaire de France, France

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ABSTRACT O_Ple2_5

Background and aim: In the late 1980s, a “large” focus of human alveolar echinococcosis (AE) was described in Zhang and Min counties, south Gansu, western China, with 71% of the families keeping dogs and a 4-23% of *E. multilocularis* prevalence in dogs (Craig et al., 1992). Multidisciplinary studies were carried out there from 1994 to 1997 and found 4.1% human AE prevalence, with peaks of 15% in some villages (Craig et al., 2000). AE was nearly 3 times higher in villages situated where areas of shrub and grassland were larger. However, populations of dogs and probably red foxes had collapsed by the early 1990s, for example only one family was found to still own a dog in Cao Tan valley. Therefore, it was inferred that human AE cases detected had been infected before the definitive host populations collapsed. From those observations, a robust transmission model involving landscape characteristics, climate, small mammal and definitive host population dynamics, was proposed which proved to be generally applicable in endemic areas of Europe as well as in most parts of China and Central Asia (Giraudoux et al., 2003; Giraudoux et al., 2013a; Giraudoux et al., 2013b). The question of the continued lack of infected definitive hosts and thus continued low/no transmission, was however pending, since Zhang and Min counties are geographically situated close to the eastern edge of the Tibetan plateau, known to be a large stable zoonotic area for AE from where reinfection is possible (dog trade, fox movements, etc.).

Material and Methods: Here we report the analysis of results obtained from a human ultrasound screening carried out in the same area in 2005-2006, and from a dog survey in May 2015 to investigate possible re-establishment of transmission after collapse of definitive host populations.

Results: No evidence was found of recent AE infection in humans in 2005-2006. Although the dog population had recovered and that forest protection and the reforestation of some areas may favour fox population growth, no evidence of infection in owned dogs was found in 2015.

Conclusion: This suggests a sustained largely environmental elimination of *E. multilocularis* transmission in this area, despite the relative vicinity of the large active foci of the eastern Tibetan plateau. The history and the socio-ecological conditions for the sustainability of continued environmental control will be discussed.
**FREE COMMUNICATIONS**

<table>
<thead>
<tr>
<th>Title</th>
<th>Added Value of Social Sciences in setting up a Control Program against Hydatidosis in Morocco.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authors</td>
<td>1. SAADI AOUATIF, AMARIR FATIMA EZZAHRA, 2. RAHELEM ABDELKEBIR, 3. FILLALI H, 4. THYS S, 5. KIRCHVINK NATHALIE, 6. MARCOTTY TANGUY, 7. BOUSLIKHANE MOHAMED, 8. OUKESSOU M, 9. SAHIBI HAMID, 10. ANTOINE-MOUSSEAUX NICOLAS.</td>
</tr>
<tr>
<td>Affiliation</td>
<td>1. Department of Pathology and Veterinary Public Health, Parasitological Unit, IAV Hassan II, Rabat, Morocco; 2. National School of Public Health, Ministry of Health, Rabat, Morocco; 3. Department of Public Health, Institute of Tropical Medicine, Antwerp Belgium; 4. Integrated Veterinary Research Unit, Department of Veterinary Medicine, University of Namur, Belgium; 5. Department of Veterinary, Biological and Pharmaceutical Sciences, IAV Hassan II, Rabat, Morocco; 6. Fundamental and Applied Research for Animals and Health (FARAH), University of Liege, Belgium.</td>
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**ABSTRACT O_Ple2_6**

**Background and aim:** Hydatidosis is highly endemic in some areas in Morocco, where its life cycle basically involves sheep, dogs and human. Despite the national program implemented since 2005, it still represents a major problem of public health due its high prevalence, morbidity as well as the economic losses that are yet to be fully evaluated. This failure calls for a full recognition of the complexity of this public health problem in the design of an improved control strategy. Complexity, in its scientific meaning, refers to a set of frameworks for analysis and action, taking account of systems properties, internal diversity and intrinsic uncertainty.

**Methods:** As part of a project exploring the applicability of sheep-vaccine-based solutions, a set of studies aims at contributing to their insertion in the wider perspective of a national control strategy. In this context, social sciences are mobilized, i.e. sociology, anthropology and socio-economics, to build up a common framework for action in a transdisciplinary perspective. The first steps of this approach involves an in-depth analysis of stakeholders, their place in the disease socio-ecological system, their degree of involvement in the past and present control strategies, their perceptions of the disease and of the past control actions.

**Results:** This communication presents the main results of a stakeholder analysis, exploring the disease system along the various interconnected socio-economic organisations, mainly connecting to the sheep value chain, the rural household and the health care system. The analysis focused on the main gaps and trade-offs in communication strategies in the Moroccan context of cultural diversity, the discussion of incentives along the sheep value chain, the place of dogs in households, as well as the link between rural households and health care systems. It proposes some tracks for further dialogue between social and health scientists in the co-construction of a response in the analysed context.
**Title**: Remapping echinococcosis in the world: epidemiology and geographic distribution of *Echinococcus granulosus* sensu lato

**Authors**: BOUFANA BELGEES

**Affiliation**: WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis, Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, ITALY

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**ABSTRACT I_Ple3_1**

**Background and Aim:** The aim of this talk is to present the current worldwide geographical and epidemiological situation for *Echinococcus granulosus* sensu lato (s.l.) taking into consideration new developments regarding the resolution of nomenclature and classification within this cryptic species. Details on how the use of genotyping allowed the acquisition of knowledge relevant to the identification and distribution of species infecting humans and animals, and how it would undoubtedly continue to do so, will be discussed. In addition, this presentation will explore how the use of DNA molecular-based methods has aided the study of polymorphisms and genetic variation of this species and allowed us to investigate its evolutionary history through the generation of hypotheses on how this zoonotic parasite may have spread.

**Materials and methods:** Several databases will be searched to retrieve publications on both historical and recent geographical distributions of *E. granulosus* (s.l.) in order to attempt to delineate up-to-date maps of *Echinococcus* spp. in the world and gain insight into the historical aspects of this distribution. Data on the epidemiology of the disease to identify *Echinococcus* species/genotype causative agents, in human and animal hosts will also be included. The genetic variation and population structure of *E. granulosus* s.l. from various regions will be discussed and compared as will the importance and relevance of the many identified haplotypes.

**Results and conclusion:** This exercise will allow us to identify and confirm the distribution of *Echinococcus* species relevant to current nomenclature as well as delineate ‘new’ species responsible for CE in human and animal hosts. It would also enable us to identify areas where the distribution of *Echinococcus* species is unknown and where more research requires to be conducted. This would ultimately enhance our understanding of local parasite transmission cycles; identify important intermediate hosts and/or wildlife cycles all of which are important for the implementation of control measures.
Molecular studies on cystic echinococcosis of camel (Camelus dromedarius) and first molecular detection of Echinococcus ortleppi from camel in Iran

SADJJADI SEYED MAHMOUD; EBRAHIMPOUR MOHAMMAD; YOSOFI DARANI HOSSEIN; NAJJARI MOHSEN.

Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

ABSTRACT O_Ple3_1

Background and Aim: Cystic echinococcosis (CE) is one of the most important zoonotic diseases, caused by different genotypes of Echinococcus spp. Camels have an important role in transmission cycle of Echinococcus granulosus especially, in desert areas. This study aimed to provide the molecular and phylogenetic data on hydatid cysts isolated from one-humped camel (Camelus dromedarius) in Iran; where G1, G3, and G6 genotypes have been reported from camel, earlier.

Methods: A total of 20 hydatid cysts samples including 18 (90%) cysts from lung and 2 (10%) cysts from liver of 56 slaughtered camels were collected from Central part of Iran. DNA was extracted from 14 fertile samples followed by molecular studies. The cytochrome c oxidase subunit 1 (COX1) and NADH dehydrogenase subunit 1 (NAD1) genes were amplified by two forward and reverse primers for COX1 gene and two forward and reverse primers for NAD1 genes, respectively. The samples were sequenced. All sequences were blasted using NCBI. Phylogenetic trees were constructed using Maximum Likelihood method. Bootstrap analysis was used to evaluate the reliability of inferred trees. Nucleotide sequences of COX1 and NAD1 genes belonged to Taenia saginata were used as out groups in the phylogenetic trees.

Results: Blast and phylogenetic analysis on sequenced genes showed the presence of G1 (28.6%), G3 (28.6%) and G6 (35.7%) genotypes in the samples. However, one sample was detected as Echinococcus ortleppi (G5) with 99% homology with G5 isolate d from camel in Egypt (AB921055) and Sudan (JX912709). It should be considered that detected G5 genotype, using COX1 gene, subsequently, was also confirmed by amplification of NAD1 gene.

Conclusion: The findings of the present study showed the presence of E. ortleppi (G5) from camel for the first time in Iran. However, other zoonotic genotypes including the G1, G3 and G6, which have been reported from camel, were also detected in the present study. Due to the transmission potential of G5 strain to human, the finding of E.ortleppi in camel should be more noticed in Iran. The data about the distribution of G5 genotype in Iran is not known. However, more studies are needed to find the distribution of G5 genotype in Iran. More molecular studies on cattle and camel hydatid cysts are needed to find the main reservoir of E. ortleppi in Iran. Moreover, molecular studies on different final hosts will evaluate the probable existence and its circulation in this country.
Title: E. multilocularis in animal hosts in Turkey

Authors: HAMZA AVCIOGLU, ESIN GÜVEN, IBRAHIM BALKAYA, ALI KURT, RIDVAN KIRMAN, MOHAMMED MEBAREK BIA, HATICE GULBEYEN, SALI YAYA, MUZAFFER AKYURT

Affiliation: Department of Parasitology, Faculty of Veterinary Medicine, Ataturk University, Erzurum 25240, Turkey; esinguven@atauni.edu.tr

ABSTRACT 0_Ple3_2

Aim: This study aimed to find out the occurrence and molecular epidemiology of Echinococcus multilocularis in animal hosts in Erzurum province, an endemic region for human alveolar echinococcosis in Turkey.

Material and Methods: To determine the prevalence of alveolar echinococcosis in final host carnivores, 440 dog and 600 fox faeces, and 30 fox carcasses were collected between February 2016 and February 2017. Besides, a total of 498 rodents were trapped in twenty counties of Erzurum province between February and December 2016. A Eurasian lynx, killed in a car crash in the north of Erzurum was taken to our laboratory and included in the scope of our project. The sedimentation and counting technique was used to reveal adult Echinococcus spp. in the intestines of foxes and lynx. The adult worms were analysed morphologically and molecularly. Faecal samples were examined by sequential sieving and flotation method to obtain taeniid eggs. During post-mortem examinations of the rodents, internal organs especially liver were analysed in terms of metacestodes. For the molecular analysis of all positive or suspected samples, DNA isolation and PCR-amplification of the partial fragment of mitochondrial 12S rRNA gene with the species-specific primers were performed. Amplified products sent to a commercial company for bidirectional sequencing.

Results: Taeniid eggs were observed in 27% (119/440) of the dog faecal samples and 24% (144/600) of fox faecal samples. Molecular analysis showed 3% (13/440) E. multilocularis infection in dogs. Molecular analyses of fox faecal samples are in progress. Eleven out of the 30 (36.7%) fox carcasses were infected with E. multilocularis. Five (1%) of the 498 rodent liver samples exhibited alveolar echinococcosis. All of the infected rodents had fertile metacestodes. The SCT results showed that the lynx was infected with E. multilocularis with a medium (745 worms) worm burden. Except taeniid eggs of foxes, all of the positive or suspected samples were confirmed to be E. multilocularis by species-specific PCR.

Conclusion: Erzurum is known to be an endemic region for AE based on human cases. With this study, the preliminary findings showed that the disease is present and prevalent in varying rates in different animal hosts in Erzurum region. This study describes first findings of E. multilocularis in dogs and rodents in Turkey. Besides, it represents the first record of E. multilocularis in a Eurasian lynx in Turkey and also in the world to our knowledge. The identification of these hosts increases the knowledge about the life cycle of the parasite in Turkey.

This work was supported financially by a grant (115S420) from the Scientific and Technical Research Council of Turkey (TUBITAK).
### ABSTRACT O_Ple3_3

**Background and Aim:** Cystic echinococcosis (CE) is a zoonosis caused by the tapeworm *Echinococcus granulosus sensu lato*. The lifecycle of the parasite is mainly domestic, requiring dogs as definitive hosts and livestock species as intermediate hosts. Although human cystic echinococcosis is a high public health priority in the Republic of Moldova, the rare animal data available concerns only infection in cattle.

**Methods:** Slaughterhouse surveillance was performed in order to evaluate the prevalence of CE in livestock originating from private households and from collective farms. Additionally, random sampling of livestock and human cases of CE were used to perform molecular characterization by sequencing part of *cox1* mitochondrial gene.

**Results:** A total of 1525 cattle, 5,580 sheep and 12,700 pigs were surveyed at the slaughterhouse. The prevalence of CE was estimated at 59.3% in cattle and 61.9% in sheep. All CE case in sheep and cattle were identified as *E. granulosus sensu stricto*. The level of infection generally appeared to be higher in private households than in collective farms. The commonly used intensive farming specific to the public sector leads to reduced exposure to the parasite, as opposed to the private sector where pasturage and free ranging are predominantly practiced. Furthermore, animals from collective farms are mainly slaughtered under strict veterinary supervision, which does not occur in the private sector where home-slaughtering is generally practiced. Since independence was proclaimed in the Republic of Moldova in 1991, the proportion of animals raised in the public and private sectors has completely reversed, with about 95% of animals now being raised in the private sector. The presence of numerous surrounding private households may explain the high prevalence in collective farms, despite the application of control measures.

**Discussion and Conclusion:** Although no CE infection was observed in pigs, our sampling concerned only industrial pig-farming complexes with indoor intensive housing. Ten samples of CE cases in pig were later collected by an additional sampling in pigs from smaller farms and revealed to be all due to *E. canadensis* G6-7. Molecular characterization of human CE cases has resulted to the identification of *E. granulosus sensu stricto* for 10 patients but also *E. canadensis* G6-7 for one. The high zoonotic potential of both *E. granulosus sensu stricto* and *E. canadensis* G6-7 leads to complete the understanding of the epidemiology of *E. granulosus s.l.* in Moldova in order to implement control and preventive measures specifically targeting farms in the private sector.
Title: Genetic microvariations within *E. ortleppi* and *E. canadensis* G6/7

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**ABSTRACT O_Ple3.4**

**Background:** *Echinococcus ortleppi* and *E. canadensis* (G6-G8 & G10) are sister species and the most recently diverged species of the genus. *E. ortleppi* is widely distributed, but its epidemiology is little understood and intraspecific variability were unknown. The various genotypes of *E. canadensis* on the other hand are generally regarded as taxa in the process of speciation. G6 and G7 are the closest related genotypes and therefore commonly referred to as G6/7 cluster though no extensive intraspecific variation studies were done.

**Methods:** Here we describe the internal genetic and populations structures of 178 *E. ortleppi* isolates from sub-Saharan Africa, Brazil, and France, and 296 *E. canadensis* G6/7 isolates from sub-Saharan Africa, Europe, and the Middle East based on the complete mitochondrial *cox1* (1608 bp) and *nad1* (894 bp) genes.

**Results:** Polymorphism within the concatenated *cox1-nad1* of *E. ortleppi* revealed 20 haplotypes that were mainly restricted to the individual countries except one that was shared by eastern and southern Africa. These haplotypes were distributed in a divergent fashion in which the French haplotype positioned more closely to the African than to the Brazilian haplotypes. The *E. ortleppi* population in Africa seem to be experiencing an expansion characterized by a relatively high haplotype diversity despite a low nucleotide diversity. Polymorphism within the G6/7 cluster of *E. canadensis* showed 73 *cox1-nad1* concatenated haplotypes with predominant and widespread central (founder) haplotypes that formed G6 and G7 sub-clusters in star-like radiations. These sub-clustering also correlated with region and animal host origin of isolates. G6 predominated in camels of sub-Saharan Africa and the Middle East, while G7 occurred mainly in pigs in Europe. There were however several isolates from Corsica, France (pigs) and Namibia (*Oryx gazella*) that occupied an intermediary position which bridged the G6 and G7 sub-clusters. The haplotype analysis demonstrated *E. canadensis* G6 and G7 to be a single coherent genotypic entity, G6/7, that is clearly distinct from *E. canadensis* G8 & G10 in comparison. The various subpopulations of *E. canadensis*
G6/7 analyzed here were also experiencing expansion except that of Corsica which was attributed to the island isolation of the parasites.

**Conclusion:** This study has shown polymorphisms within *E. ortleppi* that defines the species, and highlights the importance of host dispersal in the distribution of *E. canadensis* G6/7, reveals its radiation centres and confirms G6/7 to be a single genotype.
A comprehensive molecular survey of *Echinococcus granulosus* in formalin-fixed paraffin-embedded tissues in human isolates in Turkey

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### ABSTRACT O_Ple3_5

**Background:** Cystic echinococcosis (CE) due to the formation of a hydatid cyst is a disease commonly seen in humans and animals that can be mortal sometimes. This disease, which is present in many countries around the world, constitutes a great problem for public health and the economy. The aim of this study was to identify *Echinococcus granulosus* genotypes in formalin-fixed, paraffin-embedded tissues.

**Methods:** Tissue samples from 70 human patients with histologically confirmed echinococcosis were analysed by direct PCR of the 12S rRNA gene and by DNA sequence analysis of the CO1 gene of *E. granulosus*.

**Results:** Of the 70 samples, 29 (41.6%) could be genotypically characterized. Specifically, 26 of 70 were positive by direct G1-3 PCR of the 12S rRNA gene, two of which were identified as G1 by additional CO1 gene sequencing. All the 44 unidentified samples underwent CO1 sequencing, which yielded one G3 and two G6 records, while the remaining 41 samples gave no or inconclusive results.

**Conclusion:** The results from the analysis of human isolates of *E. granulosus* confirmed the occurrence of G1, G3 and G6 genotypes in Turkey and indicated G1/G3 cluster (*E. granulosus sensu stricto*) as the predominant genotype.
### ABSTRACT O_Ple4_1

**Background and Aim:** *Echinococcus granulosus* is a helminth that causes a cystic hydatid disease. The cyst consists of a fluidfilled vesicle surrounded by an acellular laminated layer (LL). The latter provides a mechanical and an immunological barrier against the host immune defense. In fact, the survival and persistence of the parasite in the host involves evasion strategies that may be mediated by the LL-imunomodulatory effects. In this study, we investigate the immunomodulatory effect of both *Echinococcus granulosus* infection and the laminated layer extracted from the cyst wall on inflammatory responses in experimental model of colitis.

**Methods:** The modulatory effect of both *Echinococcus granulosus* infection and the laminated layer was analyzed on dextran sulfate sodium (DSS)-induced colitis in mice. For that purpose, nitric oxide (NO) and cytokines levels (IFN-γ, TNF-α and IL-10) were assessed. The colonic expression of inducible nitric oxide synthase (iNOS) and nuclear factor-κB (NF-κB) was examined. Moreover, colonic mucus production and histological analysis were performed.

**Results:** Our results demonstrate that *E. granulosus* infection and laminated layer pretreatment significantly improved the clinical symptoms and histological scores observed during DSS-induced colitis, and also maintained mucus production by goblet cells. Concomitantly, these pretreatments reduced NO and pro-inflammatory cytokines production and attenuated iNOS and NF-κB expression in colonic tissues.

**Conclusion:** Collectively, our data are in favour to the hygiene hypothesis and indicate that prior infection with *E. granulosus* or its cyst extract can effectively protect mice from DSS-induced colitis by modulating the inflammatory responses observed in this model of colitis. Therefore, we suggest that laminated layer has a potential value in new preventive strategies against inflammatory bowel diseases.
Larval *Echinococcus multilocularis* infection reduces Dextran Sulfate Sodium (DSS)-induced colitis in mice by attenuating Th1/Th17 mediated immune reactions.

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**Aims:** The tumor-like growth of larval *Echinococcus multilocularis* tissue (causing alveolar echinococcosis, AE) is directly linked to the nature/orientation of the periparasitic host immune-mediated processes. Parasite-mediated immune suppression is a hallmark triggering infection outcome in both chronic human and murine AE. So far, little is known about secondary systemic immune effects of this pathogen on other concomitant diseases, e.g. endogenous gut inflammation. We examined the influence of *E. multilocularis* infection on murine Dextran sodium sulfate (DSS)-induced colitis.

**Materials and Methods:** At 3 months post *E. multilocularis* infection (chronic stage), the mice were challenged with 3% DSS in the drinking water for 5 days plus subsequently with tap water (alone) for another 4 days. After necropsy, fixed tissues/organs were sectioned and stained with Hematoxylin and Eosin (H&E) for assessing inflammatory reaction. Cytokine levels were measured by flow cytometry and quantitative RT-PCR. Colitis severity was assessed (by board-certified veterinary pathologists) regarding (i) colon length, (ii) weight loss and (iii) a semiquantitative score of morphologic changes.

**Results:** The histopathological analysis of the colon showed a significant reduction of DSS-induced gut inflammation by concomitant *E. multilocularis* infection, as well as down-regulation of Th1/Th17 cytokine expression, and attenuation of CD11b+ DC maturation.

**Conclusions:** *E. multilocularis* infection markedly reduced the severity of DSS-induced gut inflammation which occurred through down-regulation of Th1/Th17 T cell responses in the colon tissue. Targeting bioactive metabolites of *E. multilocularis* could be an option to develop immunotherapy tools against colitis, and putatively also against other immune disorders that exhibit a similar pathogenesis.
## ABSTRACT O_Ple4_3

**Aims:** The effect of helminth infections on inflammatory and allergic diseases is still inconclusive. Moreover, there is considerable evidence suggesting that nitric oxide (NO) plays a significant role in the physiopathology of allergic disease. In this sense, the aim of our study is to evaluate the production of NO in Algerian patients with allergic rhinitis, and allergic asthma. Moreover, we investigated *in vitro* the immunomodulatory effect of the laminated layer (LLs, outside layer of parasitic cyst) of the helminth *Echinococcus granulosus* on NO production in PBMC from allergic patients.

**Methods:** The NO production was evaluated in plasma and culture performed with peripheral blood mononuclear cells (PBMC) from patients with allergic rhinitis, allergic asthma and healthy donors. We have also investigated the effect of LLs and IL-4 on NO production by the same cells.

**Results:** Our results revealed a significant difference (*P* <0.01) in plasmatic NO levels between the two groups of patients (with asthma and allergic rhinitis) and the healthy subjects. In addition, no statistically significant differences were found between patients with allergic asthma (severe, moderate and mild) and allergic rhinitis. Interestingly, we observed that LLs reduced NO production *ex vivo*. Indeed, we reported a strong significant decrease (*p* <0.0001) of NO level in PBMC culture compared to the LLs-untreated culture. This result was confirmed using IL-4 which indicated the same effect such as LLs.

**Conclusion:** Our data confirm the implication of NO in the pathogenesis of allergic asthma and allergic rhinitis. They also support the hygienic hypothesis suggesting that *Echinococcus granulosus infection* like other helminths prevents and/or modulates inflammation during inflammatory diseases like asthma. The potential therapeutic or preventive effect of the Laminated Layer in allergic diseases remains to be investigated in a mouse model of allergic diseases.
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<th>Title</th>
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<td>Authors</td>
<td>AMRI MANEL, BOUAZIZ SAMIA, TOUIL-BOUKOFFA CHAFIA</td>
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**ABSTRACT O_Ple4_4**

**Background and aim:** *Echinococcus granulosus* infection is among the most common parasitic infections worldwide. It usually manifests as unilocular cyst(s) surrounded by an acellular laminated layer (LL). This layer provides a physical protection to the living part of the cyst including the protoscoleces. In this study, our aim is to investigate the immunomodulatory effect of LL on macrophages phenotype.

**Methods:** We examined the effect of LL extract (LLs) on parasite survival in monocytes-protoscoleces cocultures. Moreover, the expression of some markers of M1/M2 macrophages was analyzed. In this context, NOSynthase 2, Arginase and NADPH oxidase activities were measured. The involvement of mannose receptor (MR), TGF-β and PPAR-γ (Peroxisome Proliferator-Activated Receptors) was also tested using Mannan (MR antagonist), Anti-TGFβ and T007 (PPAR-γ antagonist). Finally, TLR2, CD14 and CD23 expression was evaluated using flow cytometric immunoassay.

**Results:** We observe that LLs increases protoscoleces survival *in vitro*. Furthermore, LLs inhibits NOS2 activity and enhances Arginase activity concomitantly. MR and TGF-β seem to be involved in these effects. Moreover, T007 significantly abolish LL inhibitory effect on NADPH oxidase suggesting PPAR-γ involvement in this effect. Finally, LLs increases TLR2 and CD14 expression and decreases CD23 expression by monocytes.

**Conclusion:** Our finding suggests that the laminated layer of *Echinococcus granulosus* induced M2 phenotype allowing parasite survival. The down-regulation of this pathway constitutes an important issue to address during anti-hydatic treatment design.
**ABSTRACT O_Ple4_5**

**Background and Aim:** The *Echinococcus* genomes revealed that these parasites must strongly rely on nutrients from their hosts, as they cannot synthesize amino acids, lipids and nucleotides *de novo* (Tsai et al, 2013, Zheng et al, 2013). However, this host dependency has not received further attention as a potential target for starving the parasites from essential nutrients. Several previous studies have identified metabolites and proteins of the *Echinococcus* metacestode fluid, but information on nutrient uptake and release by the parasite is scarce, if not lacking.

**Methods:** We have applied *in vitro* and *in vivo* models of alveolar echinococcosis to study the nutrients *E. multilocularis* is consuming and releasing in the form of small metabolites. In the *in vitro* model, parasite metacestode vesicles were grown in feeder cell-spent culture medium. The methods applied for metabolite identification are based on non-targeted, label-free nuclear magnetic resonance (NMR, 600 MHz).

**Results:** Principal component analysis revealed that the parasite-incubated media were clearly separated from control media. Statistical analysis identified peaks that were significantly changed upon parasite-incubation, and thus either consumed or released by the parasite: Glucose and the citric acid metabolite alpha-ketoglutarate were strongly consumed by the parasite. A variety of amino acids were detected that were consumed (e.g. threonine) or released (e.g. glycine). Moreover, products of the mitochondrial respiration chain and end products of fermentation were released, most pronounced succinate, alanine, acetate, malate and fumarate. Interestingly, also metabolites involved in the lipid metabolism were consumed by the parasite, and they will be further characterized.

**Conclusion:** NMR analysis of parasite-interacted culture medium allowed the identification of various substances that are released or consumed by the parasite. The effects these releases could have on hepatocytes will be further investigated. The *in vitro* findings of the NMR analysis are currently being compared to the composition of *E. multilocularis* vesicle fluid, as well as changes in metabolite levels in urine, plasma and liver of *E. multilocularis*-infected mice, and a first overview will be given at the conference.
**Title**
Eg-CLP1: an *Echinococcus canadensis* cathepsin-L, potentially involved in cyst formation

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**ABSTRACT O_Ple4_6**

**Background and Aim:** The lifecycle of the parasite *Echinococcus* spp. is known since 19th century. Some of their stages have been extensively studied, while others still remain poorly understood. The bibliography about cyst formation is narrowed to few articles based on morphological features, but to date, no molecular characterization has ever been reported. Several molecules, particularly cathepsins, have been found in parasites, involved in roles of extracellular matrix disruption in migration trough tissues or excystment. The aim of the present work was to identify and characterize cysteine peptidases that could eventually be involved in cyst formation.

**Material and methods:** The protoscolices from cysts of pig livers were aseptically collected, washed several times with PBS, and later pepsinized. The RNA was extracted from protoscolices, using Trizol reagent protocol and employed to construct cDNA libraries. Genomic DNA was also extracted from protoscolices, using Trizol protocol. Several PCR reactions were performed with sets of primers designed to amplify a generic cystein peptidase conserved region and a cathepsin from *E. multilocularis*, using cDNA libraries previously obtained and genomic parasite DNA as template.

The products were sequenced. These sequences were used to perform several bioinformatic studies, including sequence similarity search (BLASTn and tBLASTp), conserved domain search (CDS, Pfam, Prosite), cladogram analysis (Mega 6.0), with similar sequences from related parasites and *E. granulosus* hosts. Tridimensional modelling of translated sequences (Swiss-Model) and intron-exon prediction for PCR product sequence from genomic DNA (Genscan) was also done.

Synthetic DNA for recombinant expression of the obtained putative protein was cloned and expressed in *E. coli*. The protein was purified under denaturing conditions, and refolded by rapid dilution. Catalytic activity was determined by fluorescent substrate processing, with and without the generic cathepsin inhibitor E-64. The purified recombinant protein was employed for immunization of rabbits. Studies on protein localization at interphase parasite-host are being performed.

**Results:** Several fragments were amplified in PCRs (longest one named Eg-CLP1). Their sequences yielded similarity with cathepsins-L from several related organisms, as *Taenia*, *Spirometra*, *Hymenolepis* and *E. multilocularis*. Conserved domain search allowed the identification of a peptidase C1A domain, and inhibitor I29 in the case of the larger fragment. Cladogram analysis also grouped the translated sequence of Eg-CLP1 in clades with several *E. multilocularis* and *E. granulosus* cathepsin sequences from GenBank. This clade shared branch with *Fasciola hepatica*. 

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cathepsins, and was more dissimilar to other taenias and hosts cathepsins. Structural analysis of 3D models showed the catalytic triad conserved in spatial structure. The catalytic cleft was also present. Recombinant protein showed catalytic activity in good agreement with cathepsin L.

**Conclusion:** We found a molecule with sequence, conserved domains, 3D simulated structure and similarity by cladograms that resulted compatible with a cathepsin L. Same compatibility was showed by recombinant protein in catalytic activity and inhibition. Similar molecules of *E. multilocularis* showed collagenase and elastase activity. Several studies are currently underway to determine whether different protein components of extracellular matrix can be degraded by recombinant Eg-CLP1 and to estimate kinetic parameters for the hydrolysis of typical cathepsin L substrates by this enzyme.
### Title
Immunohistochemical observation of the inflammatory cell infiltration in adventitia of human hydatid cysts

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**ABSTRACT O_Ple4_7**

**Background:** Human cystic echinococcosis (CE) is a parasitic disease with many immunological aspects which need to be studied. The aim of the present study was to evaluate the local immune cell infiltration in human CE caused by the *Echinococcus granulosus* G1, G2 and G3, by identifying their subtypes using immunohistochemistry (IHC) method.

**Materials and methods:** In this cross sectional study 50 surgically removed hydatid cysts samples belonging to the G1, G2 and G6 genotypes and their surrounding tissue were collected from humans referred to Al Zahra Hospital, Isfahan, Iran. The fertility of 44 liver hydatid cysts was also defined. IHC was performed on the pre-cyst of 44 liver, five lung and one kidney hydatid cysts using CD3, CD19, CD8, CD4, CD68, CD56, Ki-67 and Foxp3. The eosinophils were also studied by histopathological staining. The results were then reported by observation and counting the positive and negative inflammatory cells and compared to hepatocellular carcinoma (four samples) and chronic hepatitis (four samples) as the controls. Data was analyzed using SPSS software.

**Results:** All of the 44 liver cysts were defined as fertile. Considering the biomarkers, there were positive cells for CD3+ T cells, CD19+ B cells, CD8+ cytotoxic T cells, CD4+ helper T cells, CD68+ macrophages, and Ki-67+ proliferating cells, yet in some cases, there was no positive cells for CD56+ natural killer cells (7 CE patients) and Foxp3+ regulatory T cells (one CE and one chronic hepatitis), and also there was no eosinophils in non-CE samples, including four hepatocellular carcinoma and 4 chronic hepatitis. There was no significant difference regarding the counted cell numbers among different genotypes of the parasite and different diseases. The CD3+ T cells were predominant inflammatory cells in all groups followed by CD19+ B cells. Among the T cells, CD4+ helper T cells were predominant and the CD4+/CD8+ cell ratio in CE, HC and CH patients was 2.396±0.9, 1.931±0.62 and 1.303±0.39, respectively

**Conclusion:** Based on the results of the present study, there is no difference in the studied localized immune cell population in the peri-cyst of human hydatid cysts in the G1, G2 and G6 genotypes of *E. granulosus*. Also there is no difference in the studied infiltrated immune cells between cystic echinococcosis, hepatocellular carcinoma and chronic hepatitis.
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**Authors**
1. AMARIR FATIMA EZZAHLRA, 1. SAADI AOUATIF, 1. RHALEM ABDELKBIR,
2. SAHIBI HAMID, 2. KIRCHVINK NATHALIE, 2. SADAK ABDERRAHIM,
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**ABSTRACT O_Ple5_1**

**Background:** Hydatidosis cystic is highly endemic parasitic zoonosis in Morocco, affects humans and various animal species. This study concerned hydatid disease infestation prevalence, and shows the relationship between cyst localization in organs and the fertility of cysts as well as viability of their protoscoleces.

**Methods:** A post mortem examination of 1834 ruminants (342 cattle, 805 sheep and 687 goats) was carried out at slaughterhouses in the province of Khénifra, 926 hydatid cysts (359 cattle, 507 sheep and 60 goats) were collected for the fertility and viability test, by microscopic analysis using 0.2% eosin staining.

**Results:** Results indicated a high prevalence of *E. granulosus* accounting for 29.82% in cattle, 13.29% in sheep and 2.36% in goats, with a highly significant association (P = 0.00) between prevalence and age. The most frequent site of cysts is lung for cattle and liver for sheep and goats. Location at the right lung (54.63% cattle, 53.72% sheep and 52% goat) is slightly higher than at the left lung (45.37% cattle, 46.28% sheep and 48% goats) with a favored location at the caudal lobe for the three examined species. Cysts favored location at the right and left liver lobes was also recorded for the three species. The large-sized cysts were recorded in cattle which were found to harbor hydatid cysts than sheep and goats. The fertility percentage in cysts was very high in sheep compared to goats and cattle (liver: 52.17%, 23.81%, 5.41%; lung: 55.28%, 16.67%, and 1.61%, respectively). Protoscolex viability was high in sheep and goats compared to cattle (liver: 84.97%, 74.55%, 53.54%; lungs 78.59%, 72.62%, 45.83%, respectively) with a higher average number of protoscolex per ml in sheep (572 in liver and 738 in lung).

**Conclusion:** Among the three examined species, sheep are the most important transmission source of the disease to dogs and therefore indirectly to humans. This result may be explained by the dominance of the ovine strain in Morocco.
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<th>Title</th>
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<tr>
<td>Authors</td>
<td>1AMARIR FATIMA EZZAHRA, 1SAADI AOUATIF, 1RHALEM ABDELKBIR, 1SAHIBI HAMID, 2KIRSCHVINK NATHALIE, 2BOUSLIKHANE MOHAMMED, 3MARCOTTY TANGUY, 4ANTOINE-MOUSSIAUX NICOLAS.</td>
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**ABSTRACT O_Ple5_2**

**Background and Aim:** Hydatidosis is a parasitic disease caused by *Echinococcus granulosus*. It is one of the most frequent parasitic diseases in Morocco, and is a major public health problem, that has a considerable socio-economic impact.

**Methods:** In 2005, a national hydatidosis control program has been established to reduce the impact of the disease through the application of several prophylactic measures; and an interministerial committee formed by decision-makers from the Ministry of Health, Ministry of Agriculture and the Ministry of the Interior. This work reveals the result of the control program and presents effective intermediate solutions adapted to the Moroccan context.

**Results:** In 2007, the national disease control program was founded. The target set for 2015 was to reduce by 50% the incidence of hydatid disease, it means a rate of 2.8 cases per 100 000 inhabitants. This program was based on three axes: 1) The application of preventive measures to interrupt the life cycle within hosts and between the final host and the intermediate hosts, 2) Early detection of people with hydatid cyst, 3) The availability of appropriate legislative and regulatory arsenal. Field investigations show that, to date, these objectives are far from being achieved, as the results of a retrospective survey carried out among the various services and with the actors involved in this program, revealed that since 2007 until 2017 the incidence of the hydatid cyst in humans is maintained constant mainly in the province of Khénifra with an incidence rate of 8.62 per 100 000 inhabitants. This result is explained by the lack of coordination between the various services involved in this struggle and by the lack of awareness of the population in rural areas. As in humans, the incidence in animals is stagnant, and the prevalence is very high. Moreover, field-generated results in some slaughterhouses in Khénifra province have shown a high prevalence of *E. granulosus* infestation in animals, estimated at 29.82% in cattle, 13.29% in Sheep and 2.36% in goats and even higher prevalence in older animals, prevalence of 62.5% in cattle, and 46.72% in sheep, while in dogs the average prevalence is 23.7%, this prevalence is less than that of stray dogs that are still abundant in several sites and have easy access to infested offal at slaughterhouses. We found that in stray dogs, one dog in two hosts the parasite *E.granulosus* with a prevalence of 49%. This can only
be explained by the lack of hygiene and infrastructure in these premises and the insufficient fight against the adult stage in the definitive host.

**Conclusion:** Co-operation between the different stakeholders is recommended in order to make the right decisions in a possible new hydatidosis control program, which needs to be reinforced by new and more effective control measures, integrating the human factor that remains crucial for the success of any program. Vaccination of the intermediate host (a field trial is under way), regular monitoring of the preventive chemoprevention in the final host and even companions of dogs sterilization (to reduce the canine population) which are the main source of human and animal contamination.
FREE COMMUNICATIONS

Title | Echinococcus granulosus sensu stricto and other helminths collected from necropsied stray dogs in the Tunisian Northeast.

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ABSTRACT O_Ple5_3

Background and Aim: The aims of the study were to identify helminth species present in stray dogs from two unexplored rural areas, in northeastern Tunisia, with special reference to Echinococcus granulosus and other zoonotic helminths; to determine the prevalence and intensity of infection and to assess the effect of age and gender on the epidemiological parameters.

Methods: A total of 271 stray dogs from the Tunisian northeast areas, were shot as part of a rabies campaign carried out by the Ministry of Interior. Dogs were then identified by origin, sex, age and classified into 4 age groups. At necropsy, ligatures were made at the cranial end of the duodenum and the end of the rectum, then the entire intestinal tract was removed and frozen at -80°C for 2 weeks to inactive infectious eggs of Echinococcus sp. The intestine of each dog was defrosted, opened and its contents and scrapings of the mucosa washed. The solution was allowed to stand and the sediment was examined for the presence of helminth parasites. Differentiation of cestodes species was based on hook length and the size and morphology of the proglottids. The total number of Echinococcus sp. harboured by each dog was determined. Nematodes were clarified in chloral-lactophenol solution (44%) for morphological identification and enumeration. DNA extracted from Echinococcus granulosus adult tapeworms retrieved from 10 infected dogs, was used for genotyping. The generated nucleotide sequences were compared to the Bowles et al. (1992) G1-G3 genotype sequences.

Results: A global prevalence of 98.89% and a mean intensity of 87.62 worms per infected dog were recorded. Dogs were infected with a prevalence of 97.04% for cestodes, 83.02% for nematodes, 5.53% for trematodes and 2.21% for acanthocephalans. Sixteen helminth species were reported: Dipylidium caninum, Uncinaria stenocephala, Mesocestoides lineatus, Diplopylidium noëlleri, Toxocara canis, Taenia hydatigena, Mesocestoides litteratus, Taenia pisiformis, Echinococcus granulosus, Taenia multiceps, Macracanthorhynchus hirudinaceus, Ancylostoma caninum, Trichuris vulpis, Phagicola italica, Heterophyes heterophyes and Brachylaemus sp. Zoonotic helminths were found in 99.25% of the total infected animals. Dogs were infected with E. granulosus sensu stricto (5.16%) with the highest mean intensity (566.42 worms/dog) observed for young dogs (≥ 1 to < 2 years). The obtained nonlinear age-prevalence profile suggested that older dogs acquired immunity under the E. granulosus s.s. endemic steady state equilibrium in Tunisia.

Conclusion: This study confirmed the important role of dogs in contaminating the environment and
thus potentially influencing human health. Zoonotic helminth infections are highly prevalent in dogs in northeastern Tunisia. Detection of infection in dogs is essential for implementation of control programmes against zoonotic helminths mainly *Echinococcus granulosus* in Tunisia.
Molecular characterization of *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis* in humans and livestock from Algeria.

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**Background and Aim:** In Algeria, previous molecular epidemiological studies were conducted in animals and identified *E. granulosus* sensu stricto (s.s.) genotypes G1 and G3 from various domestic herbivore cysts and *Echinococcus canadensis* genotype G6 only from dromedary cysts. Unfortunately, molecular data on human cystic echinococcosis (CE) were limited. We implemented a large genotyping study of hydatid cysts from humans and livestock animals to specify CE’s molecular epidemiology and the genetic diversity in Algeria.

**Methods:** Fifty-four human CE cysts from patients predominantly admitted in surgical units from Mustapha Hospital, Algiers, and 16 cysts from livestock animals gathered in two geographically distinct slaughterhouses, Tiaret (North-West of Algeria) and Tamanrasset (Far South of Algeria), were collected. Molecular characterization was performed using sequencing of two mitochondrial genes, cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit I (NDI).

**Results:** 90.7% of cysts (49 samples) from human were characterized as *E. granulosus* s.s. G1; 7.4% (four samples) as *E. granulosus* s.s. G3 and 1.8% as *E. canadensis* G6 (one cyst). This molecular confirmation of *E. canadensis* G6 human infection in Algeria was observed in a Tuareg female living in a desertic area in Tamanrasset. All cysts from sheep (7 samples), cattle (4 samples), and goat (3 samples) were identified as *E. granulosus* s.s. G1 and the two cysts originating from dromedary as *E. canadensis* G6. Twenty concatenated haplotypes (COI+NDI) were characterized. Among *E. granulosus* s.s., one haplotype (HL1) was highly predominant in both humans and animals cysts (71.6%).

**Conclusion:** This study revealed main occurrence of *E. granulosus* s.s. in humans and livestock animals, with description of a predominant shared haplotype corresponding to the main worldwide observed haplotype *E. granulosus* s.s. G1. *E. canadensis* G6 was limited to South Algeria, in dromedary as well as in human.
### Title
Molecular characterization of *Echinococcus granulosus* s. l. eggs from canid faecal samples: Causes and consequences of environmental contamination in Tunisia

### Authors
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### ABSTRACT O_Ple5_5

**Background and Aim:** Tunisia is a hyper endemic country for hydatidosis. The aims of this study are to 1) assess the contamination index of *E. granulosus* s. l. eggs in canid faeces and explore factors which may explain differences between regions 2) relate the human incidence with dog contamination index; 3) and finally identify the implied *Echinococcus granulosus* species.

**Material and Methods:** 1270 faecal samples from dogs and 104 faeces from wild canids (red foxes and golden jackals) were collected from the soil in four climate zones in Tunisia. The Eg1121/1122 PCR was used to assess the presence of *E. granulosus* s. l. among taeniid egg-positive samples. The nad1 PCR-RFLP method was used to identify the *E. granulosus* species. The contamination indices (CI), climatic characteristics and livestock density of studied region were compared using the principal component analysis.

**Results:** The overall CI of dog faeces by *E. granulosus* was 25.8% and different infection levels were observed between regions (p = 0.0006). The CI distribution was independent of the presence or absence of slaughterhouses (p = 0.51) and not correlated to the location in urban or rural area (p =0.31). The human surgical incidence and the CI were not related. Only *E. granulosus* s.s. was identified but genetic diversity was observed between these isolates by the use of 1121/1122 PCR. No *E. granulosus* s. l. egg was detected in the wild canid samples although dog faeces contamination was not negligible in the same area.

**Conclusion:** The relationship between human and dog infections is difficult to trace and wild canids are unlikely to participate in echiconococcosis transmission in Tunisia. The pathogen dissemination is related neither to the presence of slaughterhouses nor to the location in urban or rural areas, but it is probably influenced by human behavior towards the infected viscera.
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<th>Title</th>
<th>Prevalence of Human Cystic Echinococcosis in pastoral communities of Kenya</th>
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<tr>
<td>Authors</td>
<td>DOROTHY KAGENDO1, JAPHET MAGAMBO, ERIC MUCHIRI, EBERHARD ZEYHLE, ERASTUS MULINGE, CECILIA MBAE, MARION WASSERMANN, PETER KERN, THOMAS ROMIG</td>
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<tr>
<td>Affiliation</td>
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**ABSTRACT O_Ple6_1**

**Background and Aim:** The aim of the study was to determine the prevalence of Human Cystic Echinococcosis (CE) in pastoral communities of Kenya.

**Materials and methods:** This study was carried out in five pastoral communities in Kenya namely Turkana, Maasai, Samburu, Tharaka and Isiolo. Using a portable ultrasound imaging machine (Titan Ultrasound system, SonoSite with a 5.2 MHz transducer), a total of 14,088 persons were examined for presence of hydatid cysts. Participation was voluntary and only for those who gave informed consent prior to examination. Information about the disease and its causative agents was provided using a chart showing pictures of different stages of the disease and the ultrasound procedure. Ultrasound examinations were performed either in a health facility or done in a school or local house behind a screen to provide privacy. Depending on the facility, study subjects were screened in a lying or standing position.

**Results:** Numbers of people scanned per community were 1,501 (Samburu), 2,577 (Maasai Mara), 6,512 (Turkana) and 1,078 (Tharaka). Prevalence of CE varied from 3.1% in Turkana, 2.2% in Tharaka, 2.1% in Isiolo, 1.7% in Samburu, to 1.2 in Maasai Mara respectively. Majority of the cysts were located in the liver (65.5%), kidneys (18.6%), abdomen (11.3%), spleen (2.7%), lungs (1.7%) and lowest numbers recorded were heart (0.2%) cysts. Classification of cysts (WHO-IGWE CE classification of Ultrasound image) varied considerably.

**Conclusion:** The low prevalence of CE in Samburu (1.7%) and Maasai Mara (1.2%) communities is surprising compared with Turkana (3.1%). This is despite higher infection rates in livestock (>25%) and having more dogs per household. In this study Tharaka and Isiolo had comparatively high numbers of human CE cases. Tharaka north is located at the periphery of Meru National park and often wild animals frequent homesteads. People in this area keep large numbers of domestic dogs to keep away wild animals at night, which could contribute to the reported relatively high number of CE cases in the area. A possible reason for the higher numbers in Isiolo (an ethnically mixed community) could be due to cultural and behavioral practices amongst the three major communities (Borana, Samburu and Turkana).
**Title**: Echinococcus species and haplotypes in dogs from four different regions in Kenya.

**Authors**: Eerastus Mulinge1, 2, Japhet Magambo, David Odongo, Sammy Njenga, Eberhard Zeyhle, Cecilia Mbae, Dorothy Kagendo, Francis Addy, Dennis Ebi, Marion Wassermann, Peter Kern, Thomas Romig

**Affiliation**: 1Kenya Medical Research Institute, Nairobi, Kenya 2University of Nairobi, Nairobi, Kenya erastusmulinge@yahoo.com

**Background and Aim**: The aim of the study was to determine the prevalence, distribution and haplotypes of Echinococcus species in dogs from four different regions in Kenya.

**Materials and methods**: A total of 1,621 dog faecal samples were collected from the environment in Turkana, Meru, Isiolo and Maasai Mara regions of Kenya. Zinc chloride sieving – flotation technique was used for detection of taeniid eggs in dog faeces. Individual taeniid eggs were picked using a micro-pipette under the aid of microscope and lysed in 0.02 M NaOH to obtain DNA. The egg lysates were subjected to a nested PCR based on the mitochondrial nad1 gene. Restriction fragment length polymorphism (RFLP) was used to identify Echinococcus species, while sequencing was applied to determine Taenia species. Nested PCR of the cox1 gene was done on taeniid lysates identified as Echinococcus spp., to establish haplotypes. The PCR products were purified and sequenced. TCS 1.21 software was used for haplotypes identification and network analysis.

**Results**: Taeniid eggs were identified microscopically in 178 of 1,621 (11%) faecal samples examined. The presence of taeniid eggs differed across the four study areas, with 2.3%, 6.8%, 8.2% and 21.6% in Meru, Maasai Mara, Isiolo and Turkana respectively. Only 685 of 3352 individual taeniid egg lysates yielded products by nested PCR. RFLP and sequencing identified 283 taeniid eggs as Echinococcus species, 357 as Taenia species and 45 eggs were not determined. Echinococcus spp. reported in this study include E. granulosus s.s. (n = 166 eggs), E. canadensis G6/7 (n= 108 eggs), E. ortleppi (n = 6 eggs) and E. felidis (n = 3 eggs), the latter being the first report in dogs worldwide. Echinococcus spp. were detected in 71 of 1,621 (4.4%) faecal samples, representing 48 (9.2%), 20 (4%), 2 (0.7%) and 1 (0.3%) in Turkana, Maasai Mara, Isiolo and Meru respectively. E. granulosus s.s. was the most abundant species in dogs; being detected in 51 faecal samples, E. canadensis G6/7 in 23, E. ortleppi in 5 and E. felidis in 2 samples. Nine faecal samples harboured more than one species: 7 contained E. granulosus s.s. and E. canadensis G6/7, 1 E. granulosus s.s .and E. ortleppi, and 1 had all three species. Sequencing of the cox1 gene revealed 13 and 6 polymorphic sites for E. granulosus s.s. and E. canadensis G6/7 respectively. A total of nine haplotypes for E. granulosus s.s. were reported, 5 of which were novel. EG01 was the most common haplotype for E. granulosus ss.. Five haplotypes were identified for E. canadensis G6/7, two of which were novel.

**Conclusion**: E. granulosus s.s. remains the most common species in dogs in Kenya. We report in...
this study the first detection of *E. felidis* from dogs. The frequent presence of *E. canadensis* G6/7 in dogs in Turkana is contrary to findings in humans where the species is rare. Mixed infections and multiple haplotypes in the same faecal samples was common in this study. This study showed the existence of both domestic and wildlife cycles of CE in dogs, a factor that need to be considered when designing surveillance and control programs.
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<th>Title</th>
<th>Echinococcosis in wildlife and livestock in Namibia</th>
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<tr>
<td>Authors</td>
<td>JULIA ASCHENBORN, ORTWIN ASCHENBORN, MARION WASSERMANN, PETER DEPLAZES, THOMAS ROMIG</td>
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<tr>
<td>Affiliation</td>
<td>Vetsuisse Faculty, University of Zurich, Zurich, Switzerland</td>
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**ABSTRACT O_Ple6_3**

**Background and Aim:** To identify the different species of *Echinococcus* affecting wildlife and livestock in Namibia, and to obtain data on transmission patterns.

**Materials and Methods:** *Echinococcus* cysts cysts were collected from wildlife hunted or culled under official projects or from permitted hunting operations. Cysts from cattle were collected in export abattoirs located in central Namibia (Okahandja and Windhoek) and in northern Namibia (Katima Mulilo). Cysts were transferred into 75% ethanol, or first frozen and later transferred into ethanol and then stored at room temperature. DNA was extracted by lysing single protoscolices or tissue fragments in 10 µl 0.02 M NaOH. Mitochondrial nad1 (894 bp) and cox1 (1608 bp) genes were amplified by nested PCR using specific primer pairs. Nested PCR amplicons of both genes were purified using High Pure PCR Product Purification Kit (Roche, Mannheim-Germany) and sequenced (GATC Biotech AG, Konstanz-Germany). Rectal faecal samples from carnivores were collected opportunistically during field immobilisation of captured animals for approved routine work in wildlife projects. Taeniid eggs were retrieved from faecal material through zinc chloride flotation, lysed as described and identified through PCR. Amplicons were sequenced (GATC Biotech AG, Konstanz, Germany) and the species determined using GENtile V1.9.4 program (Manske M., 2003, University of Cologne, Germany).

**Results:** Five species of *Echinococcus* were identified in wild mammals of Namibia: *E. granulosus* sensu stricto (s.s.), *E. felidis*, *E. equinus*, *E. ortleppi* and *E. canadensis* (G6/7). *E. canadensis* (G6/7) was found in 3/32 lions, 3/36 cheetahs, 2/30 black-backed jackals, 1/8 African wild dogs and 2/24 oryx antelopes. *E. equinus* was found in 5/32 lions, 2/30 black-backed jackals and 27/43 plains zebra. *E. granulosus* s.s. was only found in 1/8 African wild dogs. *E. ortleppi* was present in 2/24 oryx antelopes. *E. felidis* was recovered from 5/32 lions and from warthogs. Samples of 44 cattle cysts were genotyped through PCR, all belonged to *E. ortleppi* (n=44).

**Conclusion:** Data on the presence, frequency and transmission patterns of defined *Echinococcus* spp. in southern Africa are sparse and fragmentary. Our study showed an unexpected diversity of *Echinococcus* taxa in wild animals in various regions of Namibia, that indicates a complex epidemiological situation. The data are indicative for the presence of sylvatic cycles (*E. equinus*) as well as for interaction between wild and domestic transmission (*E. ortleppi*). The latter species occurs regularly at low prevalence in cattle and is the only *Echinococcus* species so far identified from Namibian livestock.
Interest of the molecular characterization of Echinococcus granulosus sensu lato to study CE epidemiology in Central Sudan

Mohamed E. Ahmed¹,², Martin P. Grobusch³, Imaeldin E. Aradaib¹,⁴

Echinococcosis Research Center, Al-Neelain Institute for Medical Research (NIMR), Al-Neelain University, Khartoum, Sudan; Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, Sudan; Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Division of Internal Medicine, Amsterdam Medical Center, University of Amsterdam, Amsterdam, the Netherlands; Molecular Biology Laboratory, Faculty of Veterinary Medicine, University of Khartoum, Sudan

Echinococcosis (Hydatid) is a prevalent disease in Sudan, incidence in human ranges 0.3-1% (Central and South North Sudan respectively). Echinococcus granulosus is the main causative parasite. A hospital based study in Sudan, showed that all human cases were genotype 6 (G6), camel strain, while the incidence in animals: 7-35% in Camels, up to 10% goats, sheep and Cattles. In Tampool central Sudan 35% of camels were affected.

Among fifty samples of Echinococcus cysts obtained from affected camels at slaughter house in Tampool, which has been diagnosed macro and microscopically as well as by PCR, a sample was found to be G5 E. ortolepi (a species usually seen in cattle) which has been confirmed by DNA sequencing. Among a same number of samples obtained from affected cattle in Khartoum central Sudan, 10% were found to be G6. The camel strain G6 is almost the only pathogen that affect human.

The Development of molecular specific tests (PCR, nested, real time and LAMP recently developed) especially for G6 is paramount important for epidemiology, diagnosis and control of disease in Human and animal as well as Phylogeny for spread of the disease in Sudan and Sub-Saharean region in Africa and the world.
FREE COMMUNICATIONS | Oral | X | Session | Ple6 | Video | Poster | X
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Title | Cystic echinococcosis in donkeys in Kenya

**Authors**: EBERHARD ZEYHLE\(^1\), ERASTUS MULINGE, CECILIA MBAE, JAPHET MAGAMBO, DOROTHY KAGENDO, TIMOTHY MUTUMA, MARION WASSERMANN, PETER KERN, THOMAS ROMIG

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**ABSTRACT O_Ple6_5**

**Background and Aim**: The aim of the study was to establish the prevalence of cystic echinococcosis in donkeys in Kenya.

**Materials and Methods**: Hydatid cysts were collected from two donkey abattoirs located in Naivasha and Mogotio. The donkeys originated mainly from Maasailand and arid and semi-arid areas in Kenya but none from Turkana. The cyst materials were preserved in 80% ethanol DNA extracted using tissue commercial kits or lysis in 0.02 NaOH. Mitochondrial Nad 1 gene was amplified in a nested PCR and genotyping was done through RFLP or sequencing. PCR products were purified using Wizard® SV Gel and PCR Clean-Up System kit and sequenced by reverse primer for the nested PCR for further identification.

**Results**: A total of 2803 donkeys, 1716 from Mogotio and 1087 Naivasha were examined for the presence of CE in various organs. Hydatid cysts or lesions were recovered from 182 donkeys, of which 142 were found in liver, 35 in lungs and 5 in kidneys. Only 4 cysts were viable while 10 were sterile and 168 were calcified. PCR products were obtained from 27 of the 29 cysts genotyped and were identified as *E. granulosus* ss (n = 22), *E. ortleppi* (n = 2), *E. canadensis* G6/7 (n = 1) and not determined (n = 4).

**Conclusion**: This is the first study in sub-Saharan Africa to describe *Echinococcus* spp in donkeys. The results show a very low prevalence in donkeys and are surprising given that majority of the animals originated from Maasailand where the prevalence in livestock is higher and dogs in the same area are reported to be infected with *Echinococcus* spp. Our findings have raised the question of donkey’s susceptibility to CE as compared to other livestock species. Due to limited home slaughter of donkeys the transmission of *Echinococcus* spp to definitive hosts seems to be low and in particular transmission of *E. equinus* explaining the rarity of this species in donkeys. Previous studies on definitive hosts from the same areas don’t report this species.
**ABSTRACT O_Ple7_1**

**Title**: Scolicidal effects of some natural drug extracts: an in vitro study

**Authors**: AISSAOUI ILHAM, MIZI ALLAOUA SOFIANE, DALIA FARID, ATTRIH MOULAHEM TAYEB.

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**Introduction**: Hydatidosis is one of the dangerous zoonotic diseases that cause serious problems for human health, as well as major economic losses for livestock industry. Due to the nature of the parasite life cycle and also the structure of the cyst in human, the control of parasite in community is difficult and its treatment has faced with a major challenge. One of these challenges is inactivating the protoscolices for treatment purposes and preventing secondary cysts. Different chemicals have been used in the treatment of cyst that most of them had serious side effects for the patient. The aim of this study was investigating the scolicidal effects of some natural drug extracts in vitro.

**Materials and Methods**: Human hydatid cysts were collected from Pediatric surgery; the cyst fluid containing live protoscolices was aspirated aseptically and stored at 4°C until use. Hydro-alcoholic extract of *Punica granatum* and *Zingiber officinale* and methanolic extract of *Allium sativum* were prepared. Different concentrations of each hydro-alcoholic extract (Pomegranate and ginger) and two concentrations (50 and 75 mg per ml) of methanolic extract of garlic were prepared and protoscolices placed into incubator at 37°C. The viability of the protoscolices was determined by eosin staining method at two times (10 and 30 minutes) for Pomegranate and ginger extracts, and three times (10, 30, 60 minutes) for garlic extract.

**Results**: The extract of ginger at the concentration of 100 mg/ml and Pomegranate extract at the concentration of 5 mg/ml leads to kill all protoscolices at 10 minutes. While garlic extract killed only 93.6% protoscolices at a concentration of 75 mg / l, after 60 min exposure.

**Conclusion**: The results are very concluding especially for ginger and pomegranate for which a further complementary study in-vivo on an animal model for the sake of evaluating the efficiency and the potential side effects of these natural scolicidal agents must be performed.
### FREE COMMUNICATIONS Table

<table>
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<th>7</th>
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<td>Authors</td>
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<td>Affiliation</td>
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**ABSTRACT O_Ple7_2**

**Background and Aim:** The present study aimed to evaluate the *in vitro* scolicidal activity of *Thymus capitatus* Hoff. et Link. essential oil against *Echinococcus granulosus* protoscoleces.

**Methods:** *Echinococcus granulosus* protoscoleces were aseptically aspirated from naturally infected lungs and livers of sheep and cattle. Different concentrations of the essential oil were evaluated and the viability of protoscoleces was assessed by 0.1% eosin staining test.

**Results:** The essential oil of *Thymus capitatus* at the concentration of 3 mg/mL was able to kill 100% of the protoscoleces after only 1 min of exposure. Furthermore, GC/MS analysis indicated that carvacrol was the major component of *Thymus capitatus* essential oil.

**Conclusion:** As far as we know, this is the first study of scolicidal activity of the essential oil of *T. capitatus*. The current investigation showed potent scolicidal effect of *Thymus capitatus* essential oil *in vitro*. However, further studies are needed to evaluate the *in vivo* efficacy and safety of this essential oil for echinococcosis treatment.
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<th>Title</th>
<th>Algerian propolis antihydatic and immunomodulatory effects.</th>
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<tr>
<td>Authors</td>
<td>DEGHBAR NAHLA¹, MEZIOUG DALILA, TILIOUA SARAH, MEDJDOUB YACINE MOULoud, TOURI KAHINA, TOUIL-BOUKOFFA CHAFIA².</td>
</tr>
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<td>Affiliation</td>
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**Background and Aim:** Cystic echinococciosis (CE) is a zoonotic disease caused by the larval stage of the cestode *Echinococcus granulosus*. This disease is endemic in many parts of the world, in particular in Algeria. Propolis is a bee product widely used in folk medicine, which is increasingly being investigated as a source of biologically active compounds. The therapeutic properties of propolis have been studied in different disease experimental models. In this study we aimed to evaluate the antihydatic and immunomodulatory effects of the Ethanolic Extract of the Algerian Propolis (EEP) on the hydatid cyst in a murine model.

**Material and methods:** BALB/c mice were inoculated intraperitoneally with viable protoscoleces (PSCs) of *Echinococcus granulosus*, obtained by aseptic puncture of fertile human pulmonary hydatid cysts. After 10 days, animals were treated daily with propolis for 3 months. Cysts development was evaluated macroscopically. The production of nitric oxide was investigated in serum. In the same way, splenic iNOS expression was assessed.

**Results:** Our results showed that oral administration of propolis in infected mice has a preventive effect on the hydatid cysts. In fact, a significant decrease on cysts weight compared to the untreated control group was obtained in propolis treatment group (p<0.05). Moreover, our finding showed that CE increases NO production and iNOS expression. This increase was significantly attenuated by propolis treatment. Remarkably, weak expression of iNOS was observed in spleen sections after immunofluorescence staining, demonstrating the role of propolis in inflammation decrease.

**Conclusion:** Our results indicate both an antihydatic and immunomodulatory effects of propolis, suggesting its potential therapeutic role against *Echinococcus granulosus* infection.
Title: Pomegranate (*Punica granatum*) peel aqueous extract alone or combined with albendazole is effective in a murine model of echinococcosis developed in Swiss mice.

Authors: MOUSSA LABSI\(^1\), IMENE SOUFLI\(^1\), LILA KHELIFI\(^1\), ZINE-CHARAF AMIR\(^2\), CHAFIA TOUIL-BOUKOFFA\(^1\)

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**ABSTRACT O_Ple7_4**

**Aim:** In our current study, we investigated the effect of pomegranate peel aqueous extract (PGE) and of PGE combined with albendazole (ABZ) on the development of secondary experimental echinococcosis and the immunomodulatory properties of PGE. We focused our attention in the analysis of liver fibrogenesis and the hepatic expression of iNOS, NF-κB, TNF-α, and CD68.

**Material and Methods:** Swiss mice were inoculated intraperitoneally with viable protoscoleces. Then, PGE was orally administered daily during cystic echinococcosis development. Cyst development and hepatic damage were evaluated macroscopically and histologically. Hepatic histological changes such as the presence of reticulin and glycoproteins were analyzed and hepatic expression of iNOS, NF-κB, TNF-α, CD68 was evaluated. In another set of experiments, after 3 months of secondary experimental echinococcosis, mice were randomly allocated into five groups: ABZ-treated group, PGE-treated group, (ABZ+PGE)-treated group, infested group, and control group. Drugs in diverse treated groups were orally administered daily during CE development for 2 months.

**Results:** It was observed that *in vivo*, after treatment of cystic echinococcosis infected mice with PGE, the rate of hydatid cyst growth inhibition was concomitant with the improvement of the histological structure of liver. A significant decrease in iNOS, TNF-α, NF-κB, and CD68 expression was observed in liver tissue of treated mice (P < 0.0001). In the second set of experiments, the association of ABZ and PGE induced a significant reduction of the rate of hydatid cyst growth inhibition in comparison with the infected or ABZ-treated groups.

**Conclusion:** Our finding indicates an antihydatid and immunomodulatory properties of PGE, suggesting its potential therapeutic role against *Echinococcus granulosus* infection. The combination of PGE and albendazole improves the antihydatid properties.
A Novel PCR-RFLP assay for Molecular Characterization of *Echinococcus granulosus* sensu lato and Closely Related Species in Developing Countries

**Aim:** The sequencing approach is the gold standard for *E. granulosus* genotyping assays. Unfortunately, developing countries do not often have access to these techniques. The aim of our study was to develop an accurate PCR-RFLP (Restriction fragment length polymorphism) method in order to identify species from *E. granulosus* s. l. and perform large-scale molecular epidemiological studies in developing countries.

**Material and Methods:** Nucleotide sequences of NADH gene of a large number of *Echinococcus* spp. and *Taenia* spp. were selected from the National Center for Biotechnology databases (Genbank™). Restriction maps were analyzed to find species-specific enzymes using the software NEBcutter V2.0. *In vitro* analyses were carried out and the nad1 gene were amplified as reported by Hüttner et al., (2008) from G1, G3, G4, G5, G6, G7 and *E. multilocularis* genomic DNAs, 30 *E. granulosus* egg isolates, 86 *E. granulosus* cysts isolates (from different hosts, localization and localities) and four FF-PETs samples. PCR products were digested by the Hinf I and Hae III restriction enzymes. The restriction fragments were separated on 2% agarose gels.

**Results:** This technique was proved useful for fresh/unfixed and FF-PET tissues. The virtual restriction patterns predicted by *in silico* tools (NEBcutter V2.0) corroborate those generated through *in vitro* analyses. This new RFLP-PCR approach permitted a differentiation between *E. granulosus* sensu stricto, *E. equinus*, *E. ortleppi*, and *E. canadensis*. This technique permitted also to differentiate between *E. granulosus* sensu lato and closely related species (*E. multilocularis* and *E. shiquicus*). However, it was impossible to discriminate among the G1 and G3 and also between G6 and G7 strains with this technique. Although DNA from *E. granulosus* eggs were successfully amplified and digested, cross-reactions between *E. granulosus* s. l. and *Taenia* spp. have limited the use of our approach to characterize eggs in definitive final host feaces. *E. granulosus* sensu stricto species were identified for all cysts and no correlation between the distribution, the localizations (liver/lungs) or the host origin has been observed using the *nad1* marker.

**Conclusion:** This approach is suitable for genotyping in co-endemic *Echinococcus* areas and for large epidemiological studies in developing countries and makes also possible retrospective studies.
on fixed collections.

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Title: Proteomic characterization of human hydatid fluids: Impact on the interaction host/Echinococcus granulosus
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ABSTRACT O_Ple7_6

Background and Aim: The proteomic characterization was performed with the aim of clarifying the host–Echinococcus granulosus interaction occurring in the larval stage of Echinococcus granulosus by comparing the protein composition of the hydatid fluids according to cystic locations and fertility status.

Methods: In our study, the proteomic analysis was conducted on pulmonary (PHF), vertebral (VHF) and paravertebral hydatid fluids (PVHF). Human cysts were obtained from three different Algerian patients. Proteins were identified by searching the NCBInr database. Host proteins were organized in groups by the PANTHER® classification system. The parasite proteins were organized in groups by gene ontology (http://www.geneontology.org).

Results: The proteomic analysis of the different hydatid fluids from the larval stage of E. granulosus revealed the presence of both host and parasite proteins. Interestingly, our results showed a difference in protein profile of the parasitic proteins depending on the location and fertility status of the hydatid fluid. Host proteins such as alpha-1-antitrypsin, antithrombin III, chitinase 3-like 1 and heat shock 70 kDa protein 6 were identified in human hydatid fluid for the first time. Various parasite metabolic enzymes, including stress-related, structural and antigenic proteins, were detected in the fertile hydatid fluids (PHF and VHF). Fertile hydatid fluid contained more proteins than the infertile fluid. Few proteins were identified in the infertile fluid, most of them were immunoglobulins. Interesting differences in the antigen composition of the human fluids were reported. Antigen B was present in the PHF cyst and antigen S was present in the VHF cyst, while antigen 5 and 14-3-3 proteins were common to both cyst locations. Identification of antioxidant proteins of both host and parasite origin highlights the impact of a stressful microenvironment on establishment of the metacestode and mechanism defence of host.

Conclusion: The characterization of new hydatid proteins involved both in the physiology of the parasite and in host tissue damage may contribute to the development of new therapeutic strategies and new diagnosis tools in hydatidosis. Our results have a potential value in new strategies to improve the therapy, the prevention and diagnosis.
## Title
Contribution of the Immunoglobulin G subclasses in the detection of post-surgical recurrences of liver hydatid cysts

### Authors
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## ABSTRACT O_Ple7_7

**Background and Aim:** Serology still faces problems during the post-surgical monitoring of hydatidosis. In fact, it is difficult, by using the classical total Immunoglobulin (Ig) G, to distinguish the antibodies in relation to the recurrence from those due to the primary extracted cysts. The use of IgG subclasses seems to overcome some of these difficulties. The aim of this study was to evaluate the contribution of IgG subclasses in the diagnosis of primary infested and relapsed hydatid cysts patients.

**Methods:** Thirty four patients with primary liver cystic echinococcosis and 34 patients with liver cystic echinococcosis recurrence after surgery were included in the study. All sera were tested by Enzyme-linked immunosorbent assay anti-hydatid antigens for specific IgG1, 2, 3, 4 subclasses. Results were analyzed by using ROC curves.

**Results:** ROC curves analysis confirmed the good performances of anti-hydatid IgG1, IgG2 and IgG4 in the diagnosis of hydatidosis. However, only IgG4 had the ability to discriminate between primary infested and relapsed groups. In fact, the sensitivity of IgG4 was statistically higher in relapsed cases group (97.1% versus 70.6%, \( p=0.008 \)). High levels of specific IgG4 antibodies probably reflect an isotype switching of the humoral response during hydatid relapse.

**Conclusion:** Immunoglobulin G4 appears as a good marker of liver hydatid cyst recurrence. It could be useful for post-surgical follow up of hydatidosis to ensure early detection of relapses.
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**Title**: Serological diagnosis of confirmed Cystic Echinococcosis patients *versus* probable cases by IAH, IgG-ELISA, IEP et IgG-IB.

**Authors**: ZAIT HOURIA, HAMRIOURI BOUSSAD.

**Affiliation**: Laboratory of Parasitology and Mycology, Mustapha University Hospital, 16000 Algiers, Algeria zaithouria@gmail.com

**ABSTRACT O_Ple7_8**

**Aim**: In this work we applied four serological tests namely, indirect hemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA), immunoblotting (IB) and immunoelectrophoresis (IEP) for the human CE immunodiagnostic. Our aim is to offer reliable laboratory results in endemic conditions which would strengthen imaging findings and that would help surgeons to take therapeutic decision during the primary infection easily.

**Material and Methods**: 277 serum samples from 277 proven or probable, without previous CE history or any parasitic treatment among a total of 482 CE patients, were included in the study. Sera were collected between 2006 and 2013 and sampled before and/or at the time of the surgery. The control sera group was used for cross-reactivity detection. The serodiagnosis was made simultaneously by IHA, IgG-ELISA, IEP et IgG-IB techniques. Statistical tests applied were sensitivity (Se), specificity (Sp), Youden index, Cohen’s kappa, LR, OR and the χ² test. The percentage was calculated with a 95% confidence interval [95% CI]. A p-value below 0.05 was considered statistically significant.

**Results**: In CE proven group, we found an overall percentage positivity by the first lines tests at 83.9% [78.4%-89.3%] and 80.3% [74.3%-86.3%] for IHA and ELISA respectively whereas confirmatory tests gave a high level percentage positivity by IB than by IEP with 80.5% [74.6%-86.5%] and 60.8% [53.4%-68.1%] respectively. In sera group from probable cases, the percentage of Se was 86.4% [79.8%-93%], 72.5% [63.8%-81.2%], 48.5% [38.8%-58.2%] for IHA, ELISA and IEP respectively. The positive results by both ELISA and IB techniques used in combination allowed us to obtain more positive result (22 additional patients) more than the HAI-IEP association. The correlation between ELISA and IB (k=0.52; p<0.000) was better than IHA and IEP (k=0.46; p<0.000) and IHA and IB (k=0.42; p<0.03). All tests gave false negative reactions ranging between 16.0% and 39.2% in proven CE group. 19.4% negative results were found by IgG-IB test. The cross-reactivity with non-hydatid sera were observed in all tests, ranging from 1% to 27%. Only one patient harboring *T. saginata* serum cross reacted with 8/12kDa band by IB. We found a Sp at 73% [64.3%-81.7%], 87% [80.4%-93.5%], 99% [97%-100%] et 99% [97%-100%] by IHA, ELISA, IEP et IB respectively. In lungs, serology was less sensitive. Our scores did not exceed a maximum positivity of 65.5-70% by IB. For the liver cyst, Se was better especially by using IB (> 93%).

**Conclusion**: Various serological tests used in association improved result quality. It is suitable to use both IHA and IgG-ELISA as IgG screening tests for their complementarity and IgG-IB as confirmatory test whatever IgG level. Nevertheless, negative results by IgG-IB cannot exclude CE.
Comparison of MRI sequences for the detection of vesicles/microcysts in Hepatic Alveolar Echinococcosis (HAE) lesions

**Background and aim:** To evaluate the detection of microcysts in HAE lesions by different MRI sequences.

**Methods:** The heavy T2 spin echo sequence, T2-SPAIR sequence and DWI (b=0mm/s², b=800mm/s²) sequence were used to examine 105 lesions in 89 patients with HAE, and to determine the identification of the parasitic vesicles in HAE lesions by three MRI sequences.

**Results:** Vesicles were identified by heavy T2 spin echo sequence in 89.88% of cases, by T2-SPAIR sequence in 60.95%; and by the DWI (b=0mm/s2) sequence in 57.14%; the number of vesicles displayed by heavy T2 spin echo sequence was higher than by other sequences. Vesicles could be found around and/or in the center of the lesion.

**Conclusion:** Firstly we found that heavy T2 spin echo sequence had a great advantage in detecting the microcysts of HAE. It has important value in evaluating the growth activity of HAE and in guiding the clinical treatment.
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<th>Title</th>
<th>Pilot study for the evaluation of morphological criteria related to Hepatic Alveolar Echinococcosis based on the EMUC-CT classification</th>
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<tr>
<td>Authors</td>
<td>1 GRÄTERTILMANN; 2 KRATZER WOLFGANG; 3 SENGÜL AYLIN; 4 HILLENBRAND ANDREAS; 3 GRÜNER BEATE; 2 SCHMIDBERGER JULIAN</td>
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**ABSTRACT O_Ple9_2**

**Aim:** Computed tomography, mostly combined with PET, provides one of the most important diagnostic tools in suspected alveolar echinococcosis (AE).

**Material and Methods:** The recently established "Echinococcus multilocularis Ulm classification for computed tomography" (EMUC-CT) depicts the various morphologies of liver lesions due to AE. The classification scheme assigns morphological criteria of hepatic AE to five groups of primary morphologies (type I-V), including subcriteria and to six calcification patterns. Aim of this pilot study was to analyze several CT-morphological criteria of AE lesions in 72 patients based on the EMUC-CT classification and to make a comparison of those criteria in the clinical course under antihelminthic medication.

**Results:** The primary morphology showed to be very constant, whereas the subcriteria exhibited varying behavior. Between the patterns of calcification only slight changings could be registered. However the degree of calcification increases considerably over time. A slight regression of size in lesions under therapy could be noted. The extent and the count of lesions differ significantly between the various types. Obvious differences among the types were revealed regarding extrahepatic as well as biliary involvement. In conclusion the study could illustrate clear connections between the different morphological criteria within EMUC-CT and depicted morphological changings of AE liver lesions in the clinical course. Especially the observations concerning the extent and count of lesions in the different types and their varying behavior regarding extrahepatic as well as biliary involvement appears to be of clinical relevance.

**Conclusion:** Larger studies should be done to reveal whether conclusions could be drawn in future concerning prognosis of the disease and therapy planning.
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Title | Experimental study on the model of hepatic alveolar echinococcosis in rats based on intravoxel incoherent motion diffusion weighted imaging

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ABSTRACT O_Ple9_3

Aim : The characteristics of intravoxel incoherent motion diffusion weighted imaging (IVIM DWI) and the dynamic evolution of IVIM DWI in secondary hepatic alveolar echinococcosis (HAE) animal models were analyzed and compared to the pathological changes at different stages of HAE.

Materials and methods : The intrahepatic injection of Echinococcus multilocularis extract suspension under open vision was used to prepare the animal model of secondary HAE in rats. The rats were screened by ultrasound at the 9th week after inoculation. 15 rats were randomly selected for MRI scanning at the 10th, 18th, and 32th week after inoculation, including conventional MRI sequence and IVIM-DWI sequence. At the end of the study, 10 rats in each group were randomly sacrificed and the experimental specimens were taken for routine HE staining, Masson staining and MVD counting.

Results : 49 (49/100) rats were successfully inoculated with alveolar hydatid, and the success rate was 49%. There were significant differences in different stages (at the 10th, 18th, and 32th week) in D value and f value of HAE lesion area, peripheral area and background liver (P<0.05). There was no significant difference in D* value between the lesion area and the peripheral area, while the D* of the background liver area was higher than that of the lesion and the peripheral area. The f value of the peripheral area of HAE was statistically significant at each stage and the f value was positively correlated with microvessel density counting. The D value was slightly different at different time points, and the early D value was slightly higher than that of the late stage. There was no statistical difference in D* value at each stage.

Conclusions : 1) The state of blood flow around the HAE lesions was different from that of the internal lesions and background liver. The f value derived from the IVIM DWI sequence can well reflect the state of blood flow in the peripheral area of the lesion. 2) The dispersion states of each region in HAE were different. With time, the D value in the peripheral area of the lesion may reflect the state of local fibrosis.
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<td>Title</td>
<td>Focused Assessment with Sonography for Echinococcosis (FASE) training course in Río Negro Province, Argentina. 17 years of its application.</td>
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<td>Authors</td>
<td>LEONARDO UCHIUMI, OSCAR PANOMARENKO, MARIO DEL CARPIO, MARIANO SOBRINO, JUAN CARLOS SALVITTI, GUILLERMO MUJICA, MARCOS SELEIMAN, MARCOS AREZO, EDMUNDO LARRIEU.</td>
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**ABSTRACT O_Ple9.4**

**Aim:** To describe implementation and results of FASE training course by a Control Program of Cystic Echinococcosis (CE) of Río Negro Province, Argentina.

**Methods:** In 2000, we designed FASE training hands on course, for general practitioners, without previous experience in ultrasound (US). Lasts 2 days and a total 20 hours of continuous medical education, with 9 theoretical modules (epidemiologic, clinical, diagnostic and treatment aspects) and 2 practical (first with a group of school children apparently healthy and a second with known patients with hydatid cysts of various types of cysts). Related to the number of participants of this course, we advise 4 to 5 physicians by 1 ultrasound equipment with an instructor. Since 2000, we conducted 15 courses in Ingeniero Jacobacci and 2 in Los Menucos, both towns in highly endemic area of Río Negro Province. Two of the courses attended by professionals from Peru and Venezuela. Also we dictate the course in the provinces of San Juan, Neuquén, Chubut, Northwest area (Jujuy, Salta, Tucumán and La Rioja) and Corrientes with more than 400 participants. After the course, the trainees were able to carry out autonomous ultrasound surveys under the supervision of the referral trainer. We have also defined a protocol treatment according to type, localization and size of the cyst of asymptomatic cases: watch and wait (only monitoring with annual ultrasound scan), antiparasitic treatment with albendazol or surgery.

**Results:** our trainees carried out from 2000 to 2016, 41813 ultrasound scans in school children and 168 (0.4%) new cases were detected. All positives cases were under protocol (watch and wait, antiparasitic treatment or surgery depending localization, type of cyst and size).

**Conclusion:** US screening for CE should be focused on schoolchildren as we have implemented. Is essential as part of control program of CE. Detection of new cases in this group means recent infection and is very important for epidemiological knowledge of disease and early, timely, and appropriate treatment to reduce morbidity and mortality in human. FASE training course has proven in our experience that US diagnosis of CE can be successfully taught to non-specialists in radiology as ultrasound in trauma. This course, for general practitioners, has allowed to screening CE in a large population in remote endemic areas with persistent levels of transmission and overcome the barrier of great distances to tertiary referral center and the shortage of radiologists in rural areas. The skill of local practitioners to screening for CE using ultrasound, allowed to treat and/or follow up locally those patients who were treated with albendazol or ultrasound monitoring indicated and refers only surgical cases. Also have saved to local residents, costly travel time and missed work and avoiding unnecessary referral to tertiary center. Ultrasound for CE screening proved to be an
efficacious and low cost intervention tool for both the community and health care system.
The natural history of cystic echinococcosis in untreated and albendazole-treated patients

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Aim: Current WHO treatment protocols for Cystic Echinococcosis (CE) are based on the WHO classification. This however, assumes the WHO classification accurately reflects the natural history of the disease. This study investigated this assumption, examining whether the WHO classification reflects the natural history of CE in untreated and albendazole-treated patients.

Materials and Methods: Data were collected during mass US screenings in CE endemic regions among transhumant populations in Kenya and Morocco. Cysts were classified using the WHO standardized US classification. Patient records occurring prior to treatment, and after albendazole administration, were selected. These included 852 paired before/after observations of 360 cysts from 257 untreated patients, and 1414 paired before/after observations of 288 cysts from 157 albendazole-treated patients.

Results: A McNemar-Bowker $\chi^2$ test for symmetry was significant ($p < 0.0001$) for the 852 observations of cysts from untreated patients. 744 observations (87.3%) maintained the same class, and 101 (11.9%) progressed in a manner consistent with the classification. Regression to the CE3B stage occurred in seven of 116 CE4 cyst observations (6.0%). A McNemar-Bowker $\chi^2$ test of symmetry was also significant ($p < 0.0001$) for the 1414 observations of cysts from albendazole-treated patients. 1236 observations (87.4%) maintained the same class, and 149 (10.5%) progressed in a manner consistent with the classification. Regression to the CE3B stage occurred in 29 of 206 CE4 observations (14.1%).

Conclusions: Significant asymmetry confirms the WHO standardized US classification’s applicability to the natural history of CE and albendazole-induced changes in cysts. These findings support the use of WHO treatment protocols: because the WHO classification reflects the natural history of CE, the use of this classification to inform the treatment guidelines and provide standardized treatment is justified. Observed regressions from the CE4 to CE3B stage may reflect the stability of CE3B cysts.
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<th>Title</th>
<th>FDG-PET/MRI in alveolar echinococcosis</th>
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<tr>
<td>Authors</td>
<td>FELIX LÖTSCH A,B, FREDRIK WANECK C, HERBERT AUER D, KLAUS KACZIREK E, GEORGIOS KARANIKAŚ F &amp; MICHAEL RAMHARTER A,B,G,*</td>
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ABSTRACT O_Ple9_6

**Background:** $[^{18}F]-2$-fluoro-$2$-deoxy-$D$-glucose-positron-emission-tomography/computed-tomography (FDG-PET/CT) imaging provides important information about the size and metabolic activity caused by *Echinococcus multilocularis* and is therefore recommended for the initial assessment and follow-up of human alveolar echinococcosis (AE). The introduction of PET-magnetic resonance imaging (PET/MRI) into clinical practice in affluent health care systems provides an alternative dual imaging modality, which has not yet been evaluated for AE.

**Material/methods:** Here we describe the initial clinical experience with comparative PET/CT and PET/MR imaging in human alveolar echinococcosis patients at an Austrian reference center. PET/CT was performed on a Siemens Biograph 64 TPTV PET/CT system. Patients were administered FDG 60 minutes prior the PET/CT examination. PET/MRI investigations were performed on a Siemens Biograph mMR PET/MRI system (Siemens Medical Solutions, Erlangen, Germany). Axial in- and opposed-phase, axial T1 VIBE native and dynamic, coronal T1 VIBE post 10ml Primovist, axial diffusion, and axial T2 HASTE sequences were obtained. The effective radiation dose for CT scans and FDG exposure were calculated, and a radiologist with in-depth expertise in human echinococcosis and nuclear medicine assessed all images. This study was a descriptive study and no formal statistical hypothesis testing was, therefore, performed.

**Results:** PET/MR imaging showed comparable diagnostic capacity for liver lesions attributable to *E. multilocularis* infection, with a discrepancy only in the assessment of calcifications in one patient. Effective doses of radiation were 30.4 to 31mSv for PET/CT, which were reduced in PET/MRI to the exposure of $^{18}$F-FDG only (4.9 – 5.5mSv).

**Conclusions:** PET/MRI provides comparable diagnostic information for alveolar echinococcosis management. The reduction of radiation exposure compared to PET/CT may be of particular importance for children and young patients not amenable for curative surgery requiring repeated long-term follow-up with dual imaging modalities. Further studies are warranted to prospectively evaluate the potential of PET/MRI in the management of human alveolar echinococcosis.
### TITLE
Evaluation of Three Serological Tests Using Native Crude Antigen in Diagnosis of Hepatic Cystic Echinococcosis Patients: correlation with ultrasound cyst classification

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### ABSTRACT_O_Ple9_7

**Aim:** Diagnosis of cystic echinococcosis (CE) in humans is not easy and depends on the diagnostic facilities available in hospitals and laboratories, as well as the anatomical localization of cysts. Imaging techniques (US, CT) and serology provide both useful and complementary information on the character of the cyst and also prognosis of disease. The liver is the first and the more frequent involved organ. The aim of this study was to evaluate three different serological tests (IHA, ELISA and WB) using native crude antigen and clinical findings regarding cyst localized in the liver and individual serological responses in patients with cystic echinococcosis.

**Material and Methods:** Sheep hydatid fluid (HF) was collected from fertile cysts obtained from slaughterhouse and used as an antigen. Forty patients who were hospitalized in Dr.Ersin Arslan Training and Research Hospital in Gaziantep, Turkey were investigated by Indirect Hemaglutination Test (IHA) and Enzyme Linked Immunosorbent Assay (ELISA). We used Western blotting analysis for retesting the seropositive serum samples detected by both IHA and ELISA tests. Serum samples from surgically confirmed CE patients from Turkey and healthy Turkish people and from 16 patients with other helminthic infections such as; fasciolosis (5 patients), trichinellosis (3 patients), toxocarosis (4 patients), leishmaniosis (3 patients), giardiasis (1 patient) were included as a control group.

**Results:** Of the 40 analyzed patients, ten (25%) were men and 30 (75%) were female. The average age was 46.97 years (s.d.; 18.95) and the youngest patient was 17 and the oldest 80 years old. The majority of the patients had single cystic lesion situated in one lobe of the liver (usually in the right lobe) (55 %), 32,5 % of patients had two cystic lesions and 12,5 % of patients had multiple cyst formations with various numbers. In all cases, ultrasound (US) examinations were positive and the size of cysts were between 2.1 cm – 12.7 cm. But only twenty-three patients of the total 40 patients were classified according to the WHO classification system based on US findings as follows: CL (1 patient), CE1 (4 patients), CE2 (7), CE3 (7), CE4 (2), CE4-CE5 (1), CE1-CE2 (1). According to the results of WB test antigens with molecular weights (MW) of 8 kDa (80 %), 12 kDa (80%), 20-22 kDa (), 22-24 kDa (97,5%), 26 kDa (97,5%), 34 kDa (%100),36-38 kDa (90%), 45-50-55 kDa (97,5%), and 60-75 kDa (97,5%) were identified. But 34, 50, and 55 kDa bands were also found in other helminthic diseases. Differences, when compared to the results of the standard EgHF-ELISA, were not significant. No serum samples from healthy control reacted with EgHF antigen.
Conclusion; The aim of this study was the diagnostic performance of crude native *Echinococcus* antigen that is easy to produce, cost-efficient tools for the serological diagnosis of echinococcosis and to assess its value in defined liver CE patients. In 23 sera, we detected bands, however none of them was specific for CE1, CE2, CE3, CE4 and CE5.
Radical surgery decreases overall morbidity and recurrence compared with conservative surgery for liver cystic echinococcosis: Evidence from a meta-regression

Background and Aim: This systematic review with meta-analysis aimed to investigate whether radical surgery (RS) for Liver Cystic Echinococcosis is superior to conservative surgery (CS) to decrease morbidity, mortality and recurrence.

Methods: MEDLINE, Embase, the Cochrane Library, Scopus, INIST, Ovid, Science direct, Google scholar, Springer link, clinical key, web of science and the grey literature databases were searched up to February 13th, 2017. Adults of either sex operated on for symptomatic but non-complicated LCE were included.

The quality of studies was assessed using the Jadad scoring system or the Methodological Index for Non-Randomized Studies index when appropriate. Meta-analysis was performed with a Mantel-Hansel method for random-effects, and meta-regression was applied when appropriate.

Results: One randomized controlled trial, two retrospective comparative studies using propensity-matching analysis for comparison and 12 retrospective comparative studies were included (6167 patients). This meta-analysis showed that there were statistically significantly fewer deep Organ/Space Surgical Site Infection: OR=0.39 95%CI [0.17, 0.85] (p=0.02), biliary leakage +/-fistula OR= 0.56 95%CI [0.36, 0.86] (p=0.009), overall morbidity: OR=0.49 95%CI [0.41, 0.58] (p=0.00001) and recurrence: OR=0.18 95%CI [0.11, 0.28] (p=0.00001) in RS compared to CS.

Conclusion: This meta-analysis showed that there were statistically significantly fewer deep Organ/Space Surgical Site Infection, biliary leakage +/-fistula, overall morbidity, and recurrence in RS compared to CS.
**FREE COMMUNICATIONS**

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<tr>
<th>Title</th>
<th>Surgical management of complicated hydatid liver cysts: about 1400 complicated cysts among 2200 CE cysts.</th>
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<tbody>
<tr>
<td>Authors</td>
<td>GRAICHI RAMZI¹ - BOUALGA OMAR¹ - BELKHAROUBI KHADIDJA¹ - IKKACHE YASSER¹ - MAMOUNI SARAH¹ - KRELIL BOUABDELLAH¹</td>
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<td>Affiliation</td>
<td>¹Service de chirurgie générale et cancérologie – CHU d’Oran ; Oran - Algérie</td>
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**ABSTRACT O_Ple10_2**

**Introduction:** Despite the unquestionable contribution of imaging in the diagnosis of the hydatid cysts, cysts are discovered at the stage of complication in more than 90% of the cases. Cystic echinococcosis (CE) remains a serious pathology, evidenced by its morbidity and especially his mortality.

**Material and methods:** This retrospective study relates on the analysis of 2200 hydatid cysts treated over a period of 50 years. There were 550 men and women 1650. The average age was 40 years and ranged from 7 to 88 years old, and a peak of frequency was observed between 25 and 35 years old.

**Results:** 67% cysts were located in the right liver, the cyst was unique in 59% of cases, location double in 20% and multiple in 21% of the cases; 42% patients had a, associated lung localization, 29 a splenic location, 26 a malignant hydatidosis, 17 a mesenteric and 9 an ovarian location.

At exploration 800 cysts were noncomplicated active CE1 type cysts. All other cases were complicated: 310 with suppuration, 1010 had a biliary fistula, broken in the biliary and 12 breaking, 67 had rupture in the peritoneum. All techniques were practised, 7 marsupialisations, 2 posadas and 2 external drainage. 14 pericystic resections, 42 ideal cystectomies, 10 left hepatectomies and 64 pericystectomies, the remaining cases (1059) had a resection of the protruding dome. It may be noted that 45 patients have been treated by coelioscopic way. Drainage was performed in 468 patients divided into 10% technique of Guedj, 24% upholstery and 60% an aspirative drainage. Plasties of fillers were performed 98 times. Treatment of biliary fistulas was more nuanced, blinded systematically before 1976, we let them open with intracystic drainage with or without biliary drainage afterwards.

Mortality is close to zero (3 deaths by pulmonary embolism) and 1 by heart attack and morbidity occurred in 35% of patients, suppuration of the residual cavity, inexhaustible biliary fistula and bleeding of the surgical site. The long term follow-up allowed us to re-operate 21% of patients for a recurrence or secondary location. The length of hospital stay increased from 6 days for simple cysts to 57 days for complicated cysts.

**Conclusion:** Hydatid cysts are often complicated and complicated cysts are those that pose the most therapeutic and post-operative problems. CE cysts must be diagnosed and treated at an early stage when a conservative treatment is sufficient.
### Title
Uncomplicated hydatid cyst of the liver: Laparoscopic treatment

### Authors
ABDENOUR BELKADI, ABDELKADER MENASRIA, ANOUAR REMINI, AISSA BENSlimane, ABDELKADER BENAOUm, HABIB LARBI, NOUreddine Chadli, NASRedine Tahlaiti, OMAR TILIOuA - MOHAMED BOuBEKuER

### Affiliation
General and Laparoscopic Surgery Department, EHU Oran, Algeria

### Background and aim:
Twenty years ago, the only recognized effective treatment for the hydatid cyst of the liver was surgery. The goal is to evacuate the cyst, neutralize the parasite and treat the residual cavity either by its suppression (radical treatment) or by resection of the protruding dome.

Two major therapeutic innovations have been added to this treatment: medical treatment (Albendazole) and puncture aspiration injection and reaspiration (PAIR) under ultrasound guidance. The other innovation is laparoscopy since the publication of François Dubois in the treatment of gallbladder lithiasis in the 1990’s.

This approach is proposed for the treatment of hydatid cyst of the liver.

Our experience concerns more than 123 patients treated by laparoscopy.

### Results:
In this video, the authors propose to illustrate in a didactic way the different operative times of per laparoscopic surgical treatment of hydatid cyst of the liver.

### ABSTRACT V_Ple10_1
FREE COMMUNICATIONS

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<th>Title</th>
<th>Authors</th>
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<th>ABSTRACT O_Ple10_2</th>
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<tr>
<td>Comparison of the short-term therapeutic effects of PTBD and ERCP in the treatment of obstructive jaundice in inoperable alveolar echinococcosis</td>
<td>WANG ZHIXIN¹,², REN LI¹,², YANGDAN CAIRANG¹,², REN BIN¹,², WANG HAIJIU¹,², ZHOU YING¹,², HOU LIZHAO¹,², LIU ZHISHENG¹,², FAN HAINING¹,²*</td>
<td>¹Department of Hepatopancreatobiliary Surgery, the Affiliated Hospital of Qinghai University, Xining 810001, China; ²Qinghai Province Key Laboratory of Hydatid Disease Research, Xining 810001, China. <a href="mailto:fanhaining@medmail.com.cn">fanhaining@medmail.com.cn</a></td>
<td>Background and Aim: Comparison of the efficacy of PTBD and ERCP in the treatment of inoperable hepatic alveolar echinococcosis with obstructive jaundice. Methods: A retrospective analysis of HAE patients with obstructive jaundice at diagnosis from January 2012 to September 2016 was performed in the Affiliated Hospital of Qinghai University. The demographic information, basal laboratory tests results, hospitalization expenses and short-term prognosis of two group patients were all collected and analyzed. Results: A total of 189 cases were collected, 41 cases met the inclusion and exclusion criteria, 23 cases of which were in PTBD group, and the other 18 cases in ERCP group. There were no differences in patients demographic characteristics, IgG, HBV infection, lesion location and size, obstruction site, lesion necrosis, N and M stage, preoperative Child-Pugh classification between two groups (P&gt;0.05). However, the preoperative jaundice degree were more severe in PTBD group than ERCP group (P&lt;0.05), lesion calcification and P1, P2 lesions were more frequent in ERCP group than PTBD group (P&lt;0.05). The general condition of the patients was better in the ERCP group than that of the PTBD group before operation. The levels of jaundice related indicators were all significantly decreased after operation (P&lt;0.05), but the extent of decrease in Total Bilirubin was more obvious in PTBD group (P&lt;0.05). There were no significant differences in ALT, AST, CHE, ALB, PT, APTT, PLT between the two groups after the procedure (P&gt;0.05). There was no statistically significant difference between the two groups in postoperative complications (P&gt;0.05).The cost of treatment in PTBD group was lower than that in ERCP group (P&lt;0.05), but the total cost of hospitalization between the two groups was not statistically different (P&gt;0.05). Conclusions: PTBD decreased bilirubin more significantly and quickly. PTBD and ERCP may be equivalent in removing jaundice, and for postinterventional complications and total cost.</td>
</tr>
</tbody>
</table>
Title: Impact of enlarged and affected lymph nodes on long-term outcome after surgical therapy of alveolar echinococcosis

Authors: ANDREAS HILLENBRAND, BEATE GRUENER, KATERINA MOURATIDOU, WOLFGANG KRATZER, TILMANN GRAETER, ANNIKA BECK, THOMAS F. BARTH, KLAAUS BUTTENSOHLEN, JULIAN SCHMIDBERGER, DORIS HENNE-BRUNS

Affiliation: University Hospital Ulm; Clinic of General and Visceral Surgery

ABSTRACT O_Ple10_4

**Aim:** Alveolar echinococcosis (AE) is a serious helminthic disease. In humans, AE affects mostly the liver, but regional hepatic lymph nodes may be also involved, demonstrating dissemination of AE originating from the liver. For this reason enlarged hepatic lymph nodes can be resected while surgical treatment. We analyzed the association of resection of enlarged and affected lymph nodes on long-term outcome after surgical therapy of patients who underwent surgery with curative intent.

**Methods:** 109 curatively operated patients between 2000 and 2017 were evaluated regarding the lymph node resection/involvement, the duration of medical therapy with benzimidazole derivatives, and the further course of AE.

**Results:** In 44 out of 109 curatively operated patients, lymph nodes were resected. Most frequently, lymph nodes were resected because they were found to be enlarged in size. Infected lymph nodes were found in 11 out of these 44 patients. A systematic lymph node dissection in the hepatoduodenal ligament was performed in 14 patients. Median size of affected lymph nodes was 1.3 cm [range: “small” to 2.3 cm], median size of non-affected lymph nodes was 1.5 cm [range: “small” to 4.5 cm]. In seven patients, lymph nodes were microscopically affected by AE. In 4 patients, lymph nodes showed small particles of *Echinococcus multilocularis* (SPEM) when stained with antibody against Em2G11.

Median follow-up was 8 years for all patients, 5 years for patients having had lymph node resection, and 4 years for patients with infected lymph nodes. Overall, recurrent disease was seen in 9 patients (9/109; 8%) after a median period of time of 1 year (range 4 month to 14 years). One patient out of 33 (1/33; 3%) with resected but negative for AE lymph nodes showed recurrent disease after 4 month. None of the 11 patients with affected lymph nodes suffered from recurrent disease.

**Conclusion:** Affected lymph nodes were found in approximately one quarter of patients with lymph node resection. Hence, lymph node infection in AE is frequent. Affected lymph nodes were not larger in size when compared to non-affected lymph nodes. It is expected that more frequent use of antibody against Em2G11 will increase detection rate of SPEMs in resected lymph nodes. Lymph node infection is not associated with recurrent disease at short term.
FREE COMMUNICATIONS

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<th>Title</th>
<th>Research on the infiltration boundary of hepatic Alveolar Echinococcosis (AE)</th>
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<tr>
<td>Authors</td>
<td>TANG YOU-YIN, ZHANG HAN-ZHI, CHEN ZHE-YU</td>
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<tr>
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<td>Department of Liver Surgery, Liver Transplantation Center, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China; <a href="mailto:story_tang@foxmail.com">story_tang@foxmail.com</a></td>
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**ABSTRACT O_Ple10_5**

**Background and Aim:** The purpose of this work was to investigate the infiltration distance of *E. multilocularis* in AE.

**Methods:** We collected 36 specimens (liver tissue) from June 2016 to July 2016, and grouped them by the resection distance of 0cm, 1cm, and 2cm into three groups equally. And we performed pathological section to detect the reticulum fibres, PCR amplification to detect the 12s rRNA and western blot test to detect the Em18 protein.

**Results:** In the histopathological pictures, we could see reticulum fibres in all 12 specimens in group A, 4 specimens in group B, and no reticulum fibres were found in group C (P<0.05). Besides, we also noticed the gene of interest in all 12 specimens in group A, in contrast with 6 specimens in group B. And we still did not see any target gene in group C (P<0.05). In the western blot test, we found the Em18 protein expressed in the entire group A specimens. For group B, only 2 specimens expressed the protein. And no specimen expressed the Em18 protein in group C (P<0.05).

**Conclusion:** There was alveolar echinococcosis lesion in the site of 1cm distance from the margin, but no lesion at the site of 2cm. So, based on the absence of any parasitic materials, the suggested safe resection distance for the AE radical resection surgery was 2cm.
### ABSTRACT O_Ple11_1

**Background:** Since December 2006, the National Commission for Zoonoses (NCZ) of the Ministry of Public Health of Uruguay has conducted a new control programme for cystic echinococcosis (CE) which employed new strategies for canine diagnosis and treatment, dog population control, diagnosis in humans, epidemiological surveillance, and health education, including community participation.

**Methods:** This control programme in Uruguay addresses the control and surveillance of the disease from a holistic perspective based on Primary Health Care, which has strengthened the community’s participation in developing and coordinating activities in an interdisciplinary manner. Similarly, the control programme that is currently implemented is based on a risk-focused approach. In addition to permanent control actions in rural areas of the country, the surveillance and control measures were focused on small villages and extremely poor urban areas. Uruguay has a geographic area of 176,215 square kilometres. This area has a mild subtropical climate, with an annual average temperature of 17ºC and an average annual rainfall of 1,250 mm. The population contained 3,286,314 inhabitants in 2011. Animal husbandry is one of the most significant industries in Uruguay constituting 4% of beef exported worldwide. According to the 2015 data, the country have 11,911,110 cattle and 6,647,372 sheep. CE is endemic throughout the country and is primarily associated with the dog/sheep cycle.

**Results:** The following actions have been developed as a part of the expansion of the control and surveillance programme: a) launch of the diagnosis in dogs performed using an ELISA test for *Echinococcus* coproantigen (CoproELISA); b) anthelminthic treatment of dog population with praziquantel, orally administered every 30 days for all dogs that were under control in rural areas; c) control of dog population introduced in 2007 by means of voluntary and free surgical castration for owned dogs; d) diagnosis in humans performed using ultrasonographic surveys; e) health education conducted through the “Working Day on Health” at public health centres, employing verbal, visual and graphic methods; f) the Ministry of Livestock, Agriculture and Fisheries supplied prevalence data on livestock, which was collected from slaughterhouses data. This allowed...
performing a trace-back analysis of the infected animals that arrived to abattoirs, which enabled the identification of the infected farms and regions. The results obtained in the different actions along this programme will be presented.

**Conclusion:** According to a PAHO/WHO Inform made in 2016, the control program developed and implemented by the NCZ has been successful and in some regions of the country it is considered that the disease is currently controlled. In these areas a change in the strategy towards the elimination phase of zoonoses it is now considered. According to this PAHO/WHO inform, since the island programs that have eliminated cystic echinococcosis (e.g. New Zealand, Tasmania) there has been no control program in the world that is approaching to the elimination phases such as the program implemented by the CNZ in Uruguay. So far, the ongoing through the NCZ has been successful, supporting the inference that Uruguay is in the right path to control the CE.
Title: Human cystic echinococcosis in Tunisia: special focus on pediatric disease

Authors: M’RAD SELIM, OUDNI-M’RAD MYRIAM, CHAABANE-BANAOUES RAJA, HIZEM AMANI, KSIA AMINE, LAMIRI RACHIDA, MEKKI MONGI, NOURI ABDELLATIF, MEZHOUD HABIB, BABBA HAMOUDA

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ABSTRACT_Ple11_2

Background and Aim: Despite the deployed prevention program, cystic echinococcosis remains an important public health problem in Tunisia. Cystic echinococcosis, which commonly starts during childhood or adolescence and described as a young adult disease, may be observed at any age. The present study is a 19 years (1999–2017) analysis of children hydatidosis in Tunisia. The aim of this work is to i) identify the species/genotypes responsible of the children disease ii) study the localization and the fertility of the hydatid cysts in function of the age and the sex of patients.

Methods: Four hundred seventy seven cysts were collected from 424 children aged 2 to 16 years and operated at Monastir teaching hospital. For each cyst, the localization and the fertility of the metacestode as well as the age, the sex and the origin of the patient are listed. Molecular identification of genotype was carried out by mitochondrial 12S rRNA gene amplification and mitochondrial cytochrome C oxidase gene sequencing.

Results: The lung was the primary localization of cyst followed by the liver. The fertility of the cyst is independent of its site or its size and no relation with the age of infected children was detected. The greatest number of cases is observed in the age-groups 4-9 years. Before 10 years the infection is more frequent in the male than in the female sex because school-age boys have more external activities than girls, with a greater promiscuity with dogs.

The most frequent species/genotype associated with hydatidosis is E. granulosus sensu stricto (G1 genotype). For two children, E. canadensis (génotype G6) and E. granulosus s.s (G3 genotype) were identified for the first time in Tunisia which highlights the role of E. canadensis as a possible source of human infection. The simultaneous presence of two distinct species, E. granulosus s.s. and E. canadensis, was observed in the liver of the same patient.

Conclusion: In Tunisia, the G6 genotype is of greater public health significance than previously believed. Further studies are now required to survey the Tunisian epidemiological situation in order to determine the local transmission patterns and design appropriate control strategies.
### ABSTRACT O_Ple11_3

**Background and Aim:** Cystic echinococcosis (CE) is endemic in many areas of South America. In this context, the South American Initiative for the Control and Elimination of CE (the Initiative hereafter) coordinates regional activities towards the enhancement and standardization of laboratory diagnostic capabilities. Here, we report the results of the first inter-laboratory exercise of 5 national laboratories in Argentina (INL-ARG), Chile (INS-CHI), Peru (SENASA and INS-PER) and Uruguay (LCN-URU), affiliated to the Ministries of Health and of Agriculture, to compare the performance of their standard tests for the detection of *Echinococcus granulosus* in dog faeces.

**Methods:** Each participating laboratory sent a total of ten positive and negative faecal samples gathered from their regular surveillance activities, to the INS-CHI. At the INS-CHI the samples were recoded, divided in aliquots to form panels of 40 samples, and shipped back to each laboratory. The true status of the samples sent back to the laboratories remained blinded. Each participating laboratory tested the panel samples using the standard methodology in each country. For copro ELISA, Argentina used the protocol described by Guarnera et al, 2000, and Uruguay that described by Morel et al, 2013. For Copro PCR, Argentina used the technique described by Cabrera et al, 2002, the rest used the technique described by Stefanic et al, 2004. The sensitivity (Se) and specificity (Sp), for both PCR and ELISA for each participant laboratory were computed together with the 95% confidence intervals (95% CI) assuming the data were obtained by binomial sampling. Concordance and the Kappa test were also computed to measure the agreement between test results. In addition, laboratories completed a questionnaire on their standard practices to help understanding possible differences in the laboratories performances.

**Results:** Laboratories names were recorded (from A to E) to preserve anonymity. For PCR, Se was low across all laboratories, with best estimate at 0.46 (0.19-0.75) and 0.13 (0.0-0.4) the lowest. For Sp, laboratories performed better. The highest value was 1 (0.78-1.00) and the lowest 0.47 (0.23-0.72). Fifty percent (0.27-0.73) of the positive samples sourced from laboratory A were properly identified by the other laboratories. In contrast, only 5% of the positive samples sourced from laboratory B were identified as positive by the other 4 laboratories. As for negative samples, 85% of the samples from laboratory A and B were properly identified as negative by the other laboratories; the lowest was 48% from laboratory C. For ELISA, laboratory B returned the best Se (0.31, CI 0.09-0.61) and Sp (0.35, CI 0.14-0.62) results. In general, concordance and Kappa values were low.

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**FREE COMMUNICATIONS**

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<th>Title</th>
<th>First inter-laboratory comparison exercise of Cystic Echinococcosis diagnosis in dog faeces in Latin America</th>
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<tr>
<td>Authors</td>
<td>JERCIC MARIA ISABEL, SANTILLAN GRACIELLA, ELOLA SUSANA, QUISPE WILLIAM, CONZA LIDIA, MOLINA FLORES BEATRIZ, GAVIDIA CESAR, CABRERA MARTA, GUERRA DOS SANTOS ALEXANDRE, LARRIEU EDMUNDO, SANCHEZ-VAZQUEZ MANUEL, MAXWELL MELODY, DEL RIO VILAS VICTOR J.</td>
</tr>
<tr>
<td>Affiliation</td>
<td>Instituto Nacional ANLIS-MALBRAN, Argentina</td>
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Conclusion: In this exercise we observed significant discordance and heterogeneity. Nevertheless, the exercise contributed critical evidence, in particular from the practices questionnaire, on which the laboratories, through the Initiative, have already began to standardize protocols. The exercise also promoted communication between laboratories, identification of capacity gaps, and discussions within the Initiative concerning the reliability of surveillance figures and comparisons between countries.
### ABSTRACT O_Ple11_4

**Background:** More than 1.5 billion people (24% of the world’s population) are infected with soil-transmitted helminths worldwide. Echinococcosis and toxoplasmosis are among the main parasitic diseases of concern in Europe according to the European Food Safety Authorities. Amongst Echinococcus species, *Echinococcus multilocularis*, causing alveolar echinococcosis, is currently a real threat to public health in Europe, with a larger endemic area than previously thought. Indirect infection of human by contact with environmental matrix may be considered to be the main sources of infection rather than direct contact with infected carnivore and their feces. Only rare data are available about presence of *Echinococcus multilocularis* in soil. Prevention of alveolar echinococcosis could be greatly improved by identification of at-risk areas.

**Methods:** Identification of soil contamination by *Echinococcus multilocularis* eggs required adaptation of method for this environmental matrix. A method combining concentration of eggs by a flotation/sieving method and their detection by real-time PCR was developed. The flotation method using 10 g soil samples either spiked with different number of *E. multilocularis* eggs for validation or soil directly collected in the environment were sampled. Each qPCR reactions were performed in duplicate and run on a RotorGene thermocycler (Qiagen). All *E. multilocularis*-positive soil-samples obtained by real-time PCR were confirmed by sequencing of second real-time PCR products using another reverse primer to obtain a longer fragment of the same gene.

**Results:** A methodical sensitivity of 100% was reached for the detection of 10 *E. multilocularis* eggs spiked in 10 grams of soil. When five eggs to one egg are spiked in 10g of soil, sensitivity decreased from 80% to 33.3%. Using 20g of soil also resulted in lower sensitivity for detecting 10 *E. multilocularis* eggs than for 10 g. In 50 kitchen gardens, 250 soil samples were collected (five by garden) and analyzed according to this method. Among garden soil samples, 26 samples (10.4%) were positive for *E. multilocularis* but at the garden level, we identified 21 kitchen gardens (42%) with at least one soil positive sample.

**Conclusion:** The availability of such a method will open new perspectives, as investigations of kitchen gardens for risk assessment have become possible. Evaluation of *E. multilocularis* in environmental samples such as soil, vegetables, fruits and water can improve our understanding of sources of human cases of alveolar echinococcosis.
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<tr>
<th>Title</th>
<th>Albendazole increases the inflammatory response and the amount of Em2-positive small particles of Echinococcus multilocularis (spems) in human hepatic alveolar echinococcosis lesions.</th>
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<tbody>
<tr>
<td>Authors</td>
<td>RICKEN FRANZ¹, NELL JULIANE¹, GRÜNER BEATE², SCHMIDBERGER JULIAN³, KALTENBACH TANJA³, BECK ANNIKA¹, KRATZER WOLGANG³, HILLENBRAND ANDREAS⁴, HENNE-BRUNS DORIS⁴, DEPLAZES PETER⁵, MÖLLER PETER¹, KERN PETER², BARTH THOMAS FRIEDRICH EBERHARD¹</td>
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<tr>
<td>Affiliation</td>
<td>ⁱInstitute of Pathology, ⁱDivision of Infectious Diseases, ⁱDepartment of Medicine I, ⁱDepartment of General and Visceral Surgery, University Hospital of Ulm, Ulm, Germany; ⁱInstitute of Parasitology, University of Zürich, Zürich, Switzerland. <a href="mailto:thomas.barth@uniklinik-ulm.de">thomas.barth@uniklinik-ulm.de</a></td>
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**ABSTRACT O_Ple12_1**

**Background and Aims:** Alveolar echinococcosis (AE) is caused by the metacestode stage of Echinococcus multilocularis. The inflammatory response to this infection is influenced by the interaction of the parasite with the host. We aimed to analyze human liver lesions infected with Echinococcus multilocularis and the changes of the cellular infiltrates during albendazole (ABZ) treatment.

**Methods:** We analyzed liver tissue samples from 8 untreated patients, 5 patients treated with two daily doses of 400 mg ABZ for up to two months and 7 patients treated for more than two months with the same ABZ therapy. A broad panel of monoclonal antibodies was used to characterize the lesion by immunohistochemistry.

**Results:** A change in the cellular infiltrate was observed between the different chemotherapy times. During the initial phases of treatment an increase in CD15+ granulocytes and CD68+ histiocytes as well as in small particles of *Echinococcus multilocularis* (spems) was observed in the tissue surrounding the metacestode. Furthermore, we observed an increase in CD4+ T cells, CD20+ B cells and CD38+ plasma cells during a longer duration of treatment.

**Conclusion:** ABZ treatment of AE leads to morphological changes characterized by an initial, predominantly acute, inflammatory response which is gradually replaced by a response of the adaptive immune system.
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<th>Title</th>
<th>Watch and wait management of inactive Cystic Echinococcosis – does the path to inactivity matter? analysis of a prospective patient cohort</th>
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<tr>
<td>Authors</td>
<td>MARIJA STOJKOVIC, KERSTIN DANIELA ROSENBERGER, FRANZISKA STEUDLE, THOMAS JUNGHANSS</td>
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<tr>
<td>Affiliation</td>
<td>Dept of Infectious and Tropical Diseases, University Hospital, Heidelberg, Germany  <a href="mailto:marija.stojkovic@med.uni-heidelberg.de">marija.stojkovic@med.uni-heidelberg.de</a></td>
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ABSTRACT O_Ple12_2

**Background and Aim:** Overdiagnosis and overtreatment are rarely discussed in the context of NTDs despite their relevance for patients under the care of health services with limited resources where the risks of therapy induced complications are often disproportionate to the benefit. The advantages of cyst staging-based management of patients with cystic echinococcosis (CE) are not yet fully explored. Questions are: Do inactive cysts (CE 4 and CE 5) need treatment and is there a difference between cysts which reach CE4 and CE5 naturally or by benzimidazole therapy?

**Methods:** Analysis of long-term follow-up data from a prospective CE patient cohort of 223 patients of a national clinical center for echinococcosis. The event of interest “relapse” was defined as the reversal of a cyst from an inactive stage (CE4, CE5) back to an active stage.

**Results:** The watch & wait (ww) group included 30 patients with 46 inactive cysts who never received medical treatment. The benzimidazole-treated (med) group included 15 patients with 17 cysts. There was no relapse in the ww-group whereas 8/17 cysts showed relapse within 18 months after treatment in the med-group. Loss to follow-up was 15.5%.

**Conclusion:** Data from the watch & wait group impressively show how stable naturally inactivated cysts are in contrast to cysts which reach inactivity through treatment with benzimidazoles. A substantial proportion of patients can be spared from treatment through cyst staging. Cysts which inactivated through a natural course do not relapse with very high likelihood. We recommend follow up of 5 years to confirm the stability.
FREE COMMUNICATIONS  Oral  X Session  Ple12  Video  Poster

Title
Launching an international study on albendazole hepatotoxicity, based on a preliminary survey in 272 patients from French and Belgian reference centers

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ABSTRACT O_Ple12_3

Background and Aim: Hepatotoxicity of albendazole (ABZ) may compromise alveolar echinococcosis (AE) patient care management, since ABZ administration is mandatory for 2 years in patients with radical resection of lesions, and often for life in all other patients; there are no alternative drugs, except mebendazole (MBZ) which shares side-effects with ABZ in most cases. In order to assess the occurrence of hepatotoxicity in patients treated with ABZ and prepare a prospective research work on this subject, we initiated a preliminary retrospective survey among French-speaking reference centers.

Materials and Methods: A questionnaire was sent to all ABZ prescribers of French-speaking AE reference centers to collect the number of patients with severe hepatotoxicity due to ABZ (i.e. increased alanine-amino-transferase ≥ 5 times the upper normal value)\textsuperscript{1,2} among those patients with current AE follow-up. Additional questions aimed at recording possible risk factors, substitution to MBZ and to evaluate the feasibility of a common prospective study.

Results: Nine prescribers from 3 regions of France (Auvergne-Rhône-Alpes: 29 patients, Bourgogne Franche-Comté: 183 patients, Grand Est: 30 patients) and 2 prescribers from 2 regions of Belgium (Brussels: 8 patients, and Liège Province: 22 patients) answered the questionnaire. Among these 272 patients with current follow-up, liver toxicity occurred in 28 cases (10%). Percentage of cases, however, varied from 3% (in Belgium) to 12 % (in France, Bourgogne Franche-Comté); among 16 AE patients with a follow-up in the city of Lyon (Auvergne-Rhone-Alpes region), no case of liver toxicity was observed, whereas in the same region, 2 among the 13 AE patients followed in smaller centers presented liver toxicity (15%). Side-effect episodes usually occurred at initiation of treatment. In 17 patients a switch to MBZ was attempted; 3/17 patients had severe hepatotoxicity again, leading to permanent benzimidazole interruption. In the center with highest number of AE patients, prevalence of ABZ hepatotoxicity concerned 5/67 patients diagnosed before 2007 (7%) and 17/116 patients diagnosed after 2007 (14%).
Conclusion: Average occurrence of liver toxicity due to ABZ in European patients with AE concerns at least 1/10 patients which may have serious deleterious consequences on their care management. However, marked differences are observed between centers. This justifies further studies on possible risk factors, including AE-related liver condition, associated diseases, immune suppression, and ABZ pharmacokinetics, relating to dosage form and/or method of drug administration at the initiation of treatment. Any AE reference center interested in such study may contact the following email address: ccoms@chu-besancon.fr.

**Title**: Explorative anti-PDL1 antibody treatment of mice infected with *E. multilocularis*.

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**ABSTRACT**

**Aim**: Alveolar echinococcosis (AE) is a severe chronic helminthic disease accidentally affecting humans, whose development rather mimics a tumour-like disease. Albendazole is currently the only available treatment to manage non-resectable AE. The PD-1/PD-L1-pathway (Programme Cell Death 1) inhibits lymphocytic proliferation in tumor development. Anti-PD-1 antibodies and knocking-out PDL-1 have been used recently as immunomodulatory agents to treat lung and kidney tumors as well as melanoma, with success¹. Two recent publications showed that PD-1/PD-L1 is being overexpressed at the chronic stage of echinococcosis²,³. Based on these data, our investigations aimed at investigating the impact of treatment by anti PD-L1 on mice infected either orally or intra peritoneally with *E. multilocularis*.

**Methods**: Assays were performed in two steps: 1) Female wild type C57/BL6-mice were orally infected with eggs of *E. multilocularis*, treated 2 weeks with anti PD-L1 (beginning 1 day before infection) and sacrificed 6 weeks post infection (early stage of infection). The number of liver lesions was assessed and flow cytometry as well as RT-qPCR were performed to determine the impact on the immune response. 2) Female wild type C57/BL6-mice were intraperitoneally infected with *E. multilocularis* metacestodes. Mice were treated one month (beginning 1 day before infection) and sacrificed 2 months post infection (early stage of chronic infection). The parasite mass was assessed and flow cytometry as well as RT-qPCR were performed to determine the impact on the immune response. In all cases, control groups of non-infected mice and of infected non treated mice were analysed in parallel to the anti PD-L1 treated group.

**Results**: Both models showed a decreased parasitic development associated with anti PD-L1 treatment (lower number of liver lesions in oral model and decreased parasitic load in intraperitoneal model). Flow cytometry results and RT-QPCR assays showed a reversal of altered cell fractions (decreased NK T cells) and immune activation (decreased IL-4 and FoxP3 response) with anti PD-L1 treatment in both models.

**Conclusion**: These preliminary results suggest that the use of anti PD-L1 as treatment of echinococcosis seems promising, putatively in addition to albendazole. Further studies are needed to confirm this hypothesis with a longer time treatment initiated at the chronic stage of infection. A clinical use of recombinant antibodies of PD-1 or PD-L1, already used in oncology could be considered as a complementary therapeutic option for patients with non resectable AE.
FREE COMMUNICATIONS  Oral  X  Session  Ple12  Video  Poster

Title  Screening the pathogen box and repurposing buparvaquone against *Echinococcus multilocularis*

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**ABSTRACT O_Ple12_5**

**Background:** Alternative treatment options against alveolar echinococcosis (AE) are urgently needed. The currently available drugs of choice albendazole and mebendazole are lacking the necessary parasiticidal effects. Although AE is considered a lethal and emerging disease, it is still too rare to catch the attention of the pharmaceutical industry. Therefore, we focus on the repurposing of drugs to find new chemotherapeutic agents against *Echinococcus multilocularis*. We are mainly interested on already marketed drugs or drug classes, or compounds from other areas of research as they are more likely to be applied in patients suffering from AE.

**Methods:** The public-private partnership organization Medicines for Malaria Venture (MMV) is freely sharing their libraries of compounds and drugs, with the aim to facilitate the discovery of new drugs against malaria and neglected diseases. Recently, they developed the so-called “Pathogen Box”, which includes 400 drug-like compounds against a wide range of various infectious diseases. To find new drugs against AE, we screened the Pathogen Box *in vitro* for compounds with activity against *E. multilocularis* metacestodes.

**Results:** Eight compounds were active after 5 days of drug-incubation at a concentration of 10 µM, and they were followed-up at lower concentrations. One compound in particular, buparvaquone, received further attention as this drug is already marketed for the use against theileriosis in cattle. Consequently, the IC₅₀ of buparvaquone against metacestodes as well as isolated germinal layer cells was determined (7.8 µM and 0.04 µM respectively). On intact metacestodes, the drug was acting parasiticidal *in vitro* down to 0.3 µM, which has never observed with any other drug. On host cells, the drug did not act cytotoxic at all tested concentrations. Additionally, ultrastructural effects of buparvaquone on metacestodes were assessed by transmission electron microscopy: at 0.3 µM, the undifferentiated cells and the mitochondria were affected; at 1 µM, signs of apoptosis such as membrane stacks could be observed; and at 3 µM, the microtriches were degenerated and the germinal layer separated from the laminated layer. Following these experiments, we performed an *in vivo* trial using mice that were intraperitoneally infected with *E. multilocularis* metacestodes. The animals were treated with buparvaquone (100 mg/kg, 3 times per week, for a duration of 12 weeks) by peroral gavage, but no reduction in parasite burden was observed. The differences between the *in vitro* and *in vivo* activity of buparvaquone could be due to its mode of action involving the oxidative phosphorylation. Therefore, the targets of buparvaquone in *E. multilocularis* are currently under further investigation.

**Conclusion:** We could demonstrate that the repurposing of drugs such as those from the MMV Pathogen Box is a promising strategy to find new compounds against AE. However, translating the
in vitro results into in vivo applications remains a major obstacle to overcome.
### Title
Activity of the anti-malarial mefloquine against *Echinococcus*

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**ABSTRACT O_Ple12_6**

**Background and Aims:** Chemotherapeutical treatment options against echinococcosis rely exclusively on benzimidazoles, which, in particular in the case of alveolar echinococcosis, do not act parasicidal and might induce severe toxicity, leaving patients with no alternative treatment options. To identify novel drugs with activity, we focus on the repurposing of already marketed drugs that we test *in vitro* and *in vivo* against *E. multilocularis*.

**Methods:** We previously proved the antimalarial mefloquine to be active *in vitro* against *E. multilocularis* metacestodes. Treatment of secondary infected mice treated intraperitoneally (25 mg/kg twice per week) led to a significant reduction of parasite growth compared to the standard treatment with albendazole. However, upon oral gavage of the same dosage, mefloquine was not active. Higher dosages of mefloquine were not applied in this study due to the risk of neurological side-effects induced by mefloquine. To improve the outcome of oral treatment with mefloquine, a dose-finding study was performed. To study the mode of action of mefloquine in *E. multilocularis*, we performed drug affinity chromatography and identified ferritin and cystatin as direct or indirect binding partners.

**Results:** The dose-finding study revealed that a dosage of 100 mg/kg body weight twice per week did not induce any adverse effects in Balb/c mice. With this treatment regime, mefloquine reduced parasite weights of intraperitoneally infected mice to a similar extent as albendazole did at 200 mg/kg and at a daily dosage. In addition to terminal parasite weight assessment after treatment, we followed parasite growth by a non-invasive ultrasound scoring system. The scoring-system showed that mefloquine, like albendazole, did not reduce the total parasite mass over treatment time, but inhibited further parasite growth *in vivo*. Currently, we are investigating the treatment efficacy of mefloquine in egg-infected mice. We also compare the drug-levels in these mice with non-infected mice by HPLC, to get more insights into the pharmacokinetics of the drug in infected animals. First results of this very recent study will be available and presented at the conference.

We are currently further investigating the mode of action of mefloquine by comparing the activities of chemical derivatives, and first results will be available at the conference.