PARTNERS:
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Dear Friends and colleagues,

On behalf of the organizers we are pleased and honored to welcome you at the XXVIth World Congress on Echinococcosis – Cystic and Alveolar Echinococcosis: Old Diseases – New Challenges, which will be held in Bucharest, Romania, during 01-03 of October 2015, in the Conferences Rooms of Phoenicia Grand Hotel. The Congress is organized under the patronage of The International Association of Hydatidology (IAH), the Romanian Association of Hydatidology (RAH), “Carol Davila” University of Medicine and Pharmacy, Bucharest and Colentina Clinical Hospital.

Echinococcosis, transmitted from dog to man, is very frequent in the World and, as only a part of the number of known cases are declared, we cannot give a correct estimation of the proportions of this infection. At the same time, we are noticing an alarming increase of Alveolar Echinococcosis cases, transmitted from savage carnivorous, and with a more severe evolution compared with Cystic Echinococcosis. That is why, we consider that the development of this scientific event will raise the interest of all well-known specialists in the field, from all over the World, but, also, of our young colleagues, which will have the opportunity to learn from the experience of these specialists and, at the same time, will be able to present their work and the most recent results of their research.

The Congress is addressing to both human doctors (parasitologists, infectious diseases specialists, surgeons, epidemiologists, public health, general practitioners and laboratory specialists) and to veterinarians, as well as to those involved in the diagnostic of the disease in human and animal, to pharmacists and last but not the least, to the patients.

The Congress will be carried on in sessions of lectures, oral presentation of papers and debates based on the presented posters, the works being planned to be developed in plenum and, when the case, in sections or in satellite symposiums. The official language of the Congress is English.

We are thanking all of you in advance for your presence and for the presentation of high quality papers, which will lead to the success of this Congress, of a remarkable scientific and professional prestige.

Our Local Organizing Committee has prepared a wonderful social program for everyone to enjoy.

I hope that you will benefit from the educational as well as networking opportunities that the Conference offers. During your stay in Bucharest, take time to enjoy that various entertainment, cultural, recreational and culinary delights this vibrant city has to offer.

Warm welcome to Bucharest and enjoy your stay!

Warm regards,
Carmen-Michaela Cretu
President of the XXVIth World Congress on Echinococcosis
President of the Romanian Association of Hydatidology
GENERAL INFORMATION

HEALTH PRECAUTIONS

No immunizations or unusual health precautions are necessary or required. Romania has no infectious risks and there are no poisonous insects. There is no malaria in Romania.

Tap water is safe to drink, but we suggest using only bottled water, as Romania is home to more than 1/3 of the natural mineral springs in Europe. Bottled water is inexpensive and widely available.

Some Romanian bottled waters are rated the best in the world for purity and taste and are exported to many foreign countries.

The Romanian currency is the Leu. In this moment the exchange rate is of 1 Eur = 4,50 Lei and 1 US$ = 4,05 Lei.

Time: GMT +2 hours

ROMANIA

Romania is a unitary republic located in southeastern-central Europe, north of the Balkan Peninsula and on the western shore of the Black Sea. It is the seventh most populous member of the European Union (about 20 million people living inside the territory of Romania and more than 3 million abroad).

People speak Romanian, the language having the highest number of words of Latin origin among all other Romance languages. About 90% of the population are practitioners of Eastern Orthodoxy, the others being Catholics, Moslems, Jews and others.

Romania is the largest country in southeastern Europe (238,391 sq.km.) and the twelfth-largest in Europe. The land is distributed quite equally between the mountains, hills and plains. The Danube River is forming a large part of the border with Serbia and Bulgaria and flows into the Black Sea, forming the Danube Delta, the second largest and best preserved delta in Europe, being also a biosphere reserve and a biodiversity World Heritage Site.

Being placed alongside the parallel of 45° north, Romania has a temperate-continental climate, with four distinct seasons. By the beginning of October, temperature is between 10-22° C and the sun is to be seen between 07.15 H and 19.00 H. During the Congress we can have a sunny, worm period (“Indian summer”), but we can also face rains and colder days.

BUCHAREST

Bucharest is the capital city of Romania, the sixth largest city in the E.U. (2 million inhabitants).

The legend says that the place was founded on the banks of the Dâmbovița River, by a shepherd named Bucur (the name means “joy”).

Bucharest was first mentioned in documents in 1459. It became the capital of Romania in 1862 and is the centre of Romanian media, culture and art. Its architecture is a mix of historical (neo-classical), interbellum (Bauhaus and Art Deco), Communist-era and modern. In the period between the two World Wars, the city’s elegant architecture and the sophistication of its elite, earned Bucharest the nickname of “Little Paris”.

Economically, Bucharest is the most prosperous city in Romania and is one of the main industrial centers and transportation hubs of Eastern Europe. The city has big convention facilities, educational institutes, cultural venues, traditional “shopping arcades” and recreational areas.

Bucharest’s public transport system is the largest in Romania and one of the largest in Europe.

VENUE

Phoenicia Grand Hotel
87, Alexandru Serbanescu Str, Sector1, Bucharest
Tel: +4021 3000 888, 3000 890
Fax: +4021 3000 891
Email: office@phoenicia.ro; www.phoenicia.ro

Internet: Wifi access will be provided

Language: The official language of the Congress is English

Dates: 1-3 October 2015

Registration and Information Desk
Opened desk on the following dates:
30 September 2015  16.00-19.00
1-2 October 2015  8.00-18.00
3 October 2015  8.00-16.00

After the registration you will receive your badge and Congress kit. You are kindly invited to wear your badge during the Congress sessions and social events.

Opening Ceremony
Participants are invited to attend Opening Ceremony which will take place on Thursday, October 1st 2015 from 8.30-9.30 hours, Room Bucharest

Welcome Party
1 October 2015, 19.30 - 21.30 hours, COLOSSEUM Restaurant, Phoenicia Grand Hotel

Farewell Dinner
2 October 2015, Farewell Dinner, Disel Restaurant, 19.30-11.30 hours

Transport will be provided

Lunch: Is served daily between 13.00-14.00 hours

SESSIONS

You are kindly invited to be on session halls on time, as all the sessions will begin as per schedule. Eventually changes in the program, will be announced inside the session halls

Poster presentations
A different set of posters will be on display from 1-3.10.1015. Posters are displayed as per the posters number indicated in the program. Poster presenters are requested to stand by their posters during the breaks.

Jury will select the 10 best posters to be presented as oral presentations
## PROGRAM AT A GLANCE

### 01.10.2015

<table>
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<tr>
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### SESSION 2 (90 min)

**Chair:** Peter Kem (Germany); Thomas Romig (Germany)

**Cystic Echinococcosis in Sub-Saharan Africa (20 min each)**
- Species, genotypes and life cycles: news out of Africa: Thomas Romig (Germany)
- Cystic echinococcosis in Sub-Saharan Africa: New developments: Cecilia Mbae (Kenya)
- Genetic structure of Echinococcus canadensis and E. ortleppi in Sub-Saharan Africa: Francis Addy (Ghana)
- Cystic Echinococcosis in a Central African Tropical Rainforest Ecosystem – an epidemiologic Pilot Study from Gabon: Felix Lötch / Michael Ramharter (Austria)
- *Echinococcus* spp. and other taeniid cestodes in wildlife of southern Africa: Marion Wassermann (Germany)

### SESSION 3 (120 min)

**Chair:** Thomas Junghanss (Germany); Carmen-Michaela Cretu (Romania)

**Diagnosis and management of abdominal CE in humans (20 min each)**
- Serology for the diagnosis and follow up of CE: is it worth using it?: Mar Siles-Lucas (Spain)
- Up to date on the diagnosis of Cystic Echinococcosis: Nadjmiye Altitans (Turkey)
- Watch and wait for Cystic Echinococcosis: Enrico Brunetti (Italy)
- Controversies in the management of cystic echinococcosis. Is it time for a change?: Peter Chiodini (UK)

Free oral presentations (10 min each)
- IntraCystic drug concentration of Albendazole and its active metabolite: albendazole sulphoxide in human cystic echinococcosis: A systematic review: Felix Lötch (Austria)
- The relationship of *Echinococcus* and *Helicobacter pylori*: Hao Wen (China)
- Performance comparison of three rapid diagnostic tests for the seroDiagnosis of hepatic cystic echinococcosis in humans: Francesca Tamarozzi (Italy)
- Real-time Loop-Mediated Isothermal Amplification Assay for Simple and Rapid Detection of Cystic Echinococcosis, Sudan: Mohamed Ahmed (Sudan)

### SESSION 4 (120 min)

**Chair:** David Heath (New Zealand); Ioan Liviu Mitrea (Romania)

Advances in diagnosis and treatment of CE in intermediate and definitive hosts (20 min each)
- Focused assessment with sonography for Echinococcosis (FAS): training course: impact in Rio Negro Province, Argentina: Leonardo Uchiumi (Argentina)
- Treatment in definitive hosts: Vasile Cozma (Romania)
- *Echinococcus* spp. and other taeniid cestodes in wildlife of southern Africa: Marion Wassermann (Germany)
- Cystic Echinococcosis in Iran: the significance of camel-dog cycle: Majid Fasih (Iran)
- Discussions
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<thead>
<tr>
<th>Hour</th>
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<th>Topic</th>
<th>Presenters/Authors</th>
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<tr>
<td>8.00</td>
<td>SESSION 7 (120 min)</td>
<td>Room A-Bucuresti</td>
<td>Peter Deplazes (Switzerland); Diana Onac (Romania)</td>
<td>Diagnosis and management of AE</td>
<td>Comprehensive diagnosis of 112 patients (2012 to 2015); Andreas Hillebrand (Germany)</td>
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<td>Free oral presentations (10 min each)</td>
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<tr>
<td>9.00</td>
<td>SESSION 8 (90 min)</td>
<td>Room B-Tyr</td>
<td>Stadelmann (Switzerland)</td>
<td>Comprehensive diagnosis and treatment of Alveolar Echinococcosis (AE) in Ulm/Germany: A single-center experience of 112 patients (2012 to 2015): Andreas Hillenbrand (Germany)</td>
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<td>10.30</td>
<td>SESSION 9 (90 min)</td>
<td>Room C-Beirut</td>
<td>António Menezes da Silva (Portugal); Irinel Popescu (Romania)</td>
<td>Complications and treatment of abdominal CE (20 min each)</td>
<td>Andrea Di Costanzo (Italy) Metropolitan treatment of complicated hydatid cyst of the liver: Mustapha Benzakour (Morocco) Andrea Di Costanzo (Italy) Mongodi Vittorio (Italy) Surgical approach for pulmonary hydatid cysts: 30 years of experience: Sebastian Ionescu (Romania)</td>
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<td>SESSION 10 (90 min)</td>
<td>Room A-Bucuresti</td>
<td>Daniel Hegglin (Switzerland); Patricia Mihailescu (Romania)</td>
<td>Echinococcus multilocularis in the light of human-wildlife interactions: Daniel Hegglin/ Britta Stadelmann (Switzerland)</td>
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<td>SESSION 11 (90 min)</td>
<td>Room B-Tyr</td>
<td>Antonio Menezes da Silva (Portugal); Irinel Popescu (Romania)</td>
<td>Status of Neotropical Echinococcosis: Katherina Vizcaychipi (Argentina)</td>
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<td>SESSION 12 (90 min)</td>
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<td>Peter Chiodini (UK); Ioan Cordos (Romania)</td>
<td>Peculiarities of Lung CE - Experience in Sudan: Mohamed Ahmed (Sudan)</td>
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<td>Daniel Hegglin (Switzerland); Patricia Mihailescu (Romania)</td>
<td>New tools for diagnosis with a multidisciplinary approach: Ariel Na idich - Video presentation</td>
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<td>15.30</td>
<td>SESSION 14 (90 min)</td>
<td>Room B-Tyr</td>
<td>Ioan Cordos (Romania)</td>
<td>Treatment of neotropical polychistic echinococcosis: Nilton Ghiotti de Siqueira (Brazil)/Antonio Menezes da Silva</td>
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<td>16.30</td>
<td>SESSION 15 (90 min)</td>
<td>Room C-Beirut</td>
<td>Enrico Brunetti (Italy); Dominique Vuiton (France)</td>
<td>Status of Neotropical Echinococcosis: Katherina Vizcaychipi (Argentina)</td>
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<td>Status of Neotropical Echinococcosis: Katherina Vizcaychipi (Argentina)</td>
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<td>Daniel Hegglin (Switzerland); Patricia Mihailescu (Romania)</td>
<td>Treatment of neotropical polychistic echinococcosis: Nilton Ghiotti de Siqueira (Brazil)/Antonio Menezes da Silva</td>
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<td>19.30</td>
<td>SESSION 18 (80 min)</td>
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<td>Peter Chiodini (UK); Ioan Cordos (Romania)</td>
<td>Status of Neotropical Echinococcosis: Katherina Vizcaychipi (Argentina)</td>
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<td>12.30</td>
<td>Lunch / Poster viewing – Cystic echinococcosis</td>
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| 14.00 | SESSION 9 (90 min) | Chair: Peter Chiodini (UK); Ioan Cordos (Romania)  
Treatment of non-complicated Liver CE (20 min each)  
- Treatment of Liver cystic Echinococcosis - Nomenclature and therapeutic options: António Menezes da Silva (Portugal)  
- Allocation of Cystic Echinococcosis patients to treatment: Enrico Brunetti (Italy)  
- Place of non-radical surgery for cystic Echinococcosis: Mircea Beuran (Romania)  
- Percutaneous treatment of type III (CE2/3b) liver hydatid cysts: comparison results of a new modified catheterization technique (MoCAT) with PAIR and catheterization: Okan Akhan (Turkey)  
Free oral presentation (10 min)  
- Laparoscopic approach in liver hydatid cyst – possibilities and limits: Ion Cosmin Puia (Romania) |
| 15.30 | Break / Poster viewing – Cystic echinococcosis | |
| 16.00 | SESSION 10 (90 min) | Chair: Mohamed Ahmed (Sudan); Sebastian Ionescu (Romania)  
Lung and other forms of CE (20 min each)  
- Treatment of non-complicated lung cysts: Karima Achour (Algeria)  
- Peculiarities of Lung CE - Experience in Sudan: Mohamed Ahmed (Sudan)  
- Misdiagnosis of lung echinococcosis: Ioan Cordos (Romania)  
Free oral presentations (10 min each)  
- Surgical approach for pulmonary hydatid cysts - 30 years of experience: Sebastian Ionescu (Romania)  
- 200 cases clinical introduction of hepatic cystic echinococcosis’ standard surgical treatment: Ayidu Reyimu (China)  
- Application of individualized therapy in surgical treatment of hepatic hydatid disease and its clinical significance: Maimaitituexun Tuerdi (China) |
| 17.30 | IAH GENERAL ASSEMBLY | Gala Dinner |

**SESSION 13 (90 min)**  
Chair: Daniel Heglin (Switzerland); Patricia Mihaiescu (Romania)  
Ecology of alveolar echinococcosis  
Free oral presentations (10 min)  
- The zoonotic transmission of Echinococcus multilocularis in the light of human-wildlife interactions: Daniel Heglin/Britta Stadelmann (Switzerland)  
- Echinococcus multilocularis in different rodent species: heterogeneous transmission dynamics at a small spatial scale: Oliva Beerli (Switzerland)  
- Primary (oral) infection of Echinococcus multilocularis eggs in various species of ecologically relevant intermediate hosts: Ian David Woolsey (Denmark)  
- Surveillance and management of Echinococcus multilocularis in a wildlife park: Gerald Umhang (France)  
- The role of the rodent in the Echinococcus multilocularis lifecycle in Sweden: Andreea Miller (Sweden)  
- Discussions
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| 08.30 | **SESSION 15 (120 min)**<br>Chair: Benoit Combes (France); Eniko Barabas-Hajdu (Romania)  
Epidemiology and control of AE (20 min each)<br>• Epidemiology and control of AE in European countries: Bruno Gottstein (Switzerland)<br>• Current situation of echinococcosis management. Networks and improvement in China: Wen Hao (China) - 20 min<br>• Control of Echinococcus multilocularis in definitive hosts: Thomas Romig (Germany)<br>• Alveolar echinococcosis in the field. What could be offered in order to control the parasite and its main definitive hosts?: Benoit Combes (France)<br>• Towards an European database on human cases of Alveolar echinococcosis: Laurence Millon (France)  
Free oral presentation (10 min)<br>• Immunomodulation of host immunity by larval Echinococcus multilocularis: Wang Junhua (China) / Bruno Gottstein (Switzerland)  
Discussions | **SESSION 19 (120 min)**<br>Chair: Leonardo Uchiumi (Argentina), Irina Dumitru (Romania)  
Cystic echinococcosis – diagnosis and follow up<br>Free oral presentations (10 min each)<br>• Cloning and characterization of potent Kunitz type protease inhibitors from Echinococcus granulosus: Donald P McManus (Australia)<br>• Recovery indicator for human cystic echinococcosis/hydatidosis patients after surgery by evaluation of their IgG and its subclasses (IgG1 and IgG4): Saeid Mahmoud Sadjadi (Iran)<br>• Living donor liver transplantation for a complex hepatic CE: a case report from China: Shao Yingmei (China)<br>• Analysis of 105 patients with hepatic cystic echinococcosis Type 4-5: Kadieralli Aernix /Guo Yong Zhong (China)<br>• Monitoring Heracles prospective patients and prepare their registration in the European Register for cystic echinococcosis (ERCE): Gabriela-Loredana Popa (Romania)<br>• The role of the pericyst – digestive Anastomosis in the surgical treatment of the hepatic hydatid cyst: Cristian Botezatu (Romania)<br>• Minimal invasive techniques for liver hydatid disease: implementation of Heracles Project: Cosmin Alexandru Popa (Romania)<br>• Evolution of minimally invasive Surgery in the management of hepatic hydatid cyst: Fadma Adi (Morroco)<br>• Hydatidosis in the province of Catamarca, Argentina: anatomic location of the cysts and geographical distribution of cases in the period 2010-2014: Lopez Raul Alredo (Argentina)<br>• Neglected diseases returning into the attention of national public health authorities through international projects (case study HERCLES Project): Mircea-Ioan Popa (Romania)<br>• Research structure in order to improve quality of researches – Al Neelain University experience: Sara-Lavinia Brair (Sudan)  
Discussions |
| 10.30 | Break / Poster viewing - Alveolar echinococcosis | Break / Poster viewing - Alveolar echinococcosis |
| 11.00 | **SESSION 16 (90 min)**<br>Chair: Bruno Gottstein (Switzerland); Ion Cosmin Pula (Romania)  
Advances in diagnosis and treatment of AE in humans (20 min each)<br>• Immune suppression and AE in experimental animals and in humans: Dominique Vuitton (France)<br>• Advances in immunological diagnosis and follow-up of AE: Laurence Millon (France)<br>• Pitfalls in diagnosis and treatment of alveolar echinococcosis: a sentinel case series: Marija Stojkovic (Germany)<br>• A European survey of the treatment of biliary complications by per-endoscopic procedures in patients with alveolar echinococcosis: Sylvain Ambregna / Dominique Vuitton (France)<br>• Ex vivo liver resection and autotransplantation for end-stage Alveolar Echinococcosis: a case series: Hao Wen (China)  
Discussions | **SESSION 20 (90 min)**<br>Chair: Nazmiye Altintos (Turkey); Teofilia Banu (Romania)  
Diagnosis and management of CE<br>Free oral presentations (10 min each)<br>• Cystic echinococcosis in HIV infected patients: Irina Magdalena Dumitru (Romania)<br>• Evaluation of chemotherapy in treating ewes naturally infected with hydatid disease: Haitao Li (China)<br>• Investigations of generic diversity in Echinococcus granulosus sensu stricto using a new microsatellite polymorphism approach: Umhang Gerald (France)<br>• Strain characterization of E. granulosus bovine cyst and serological interleukin levels in hydatidosis and diamatosis infected cattle: Felipe Correa (Chile)<br>• Surgical and molecular evaluation of pediatric hydatid cyst cases and some risk factors in Eastern Turkey: Sami Simsek (Turkey)  
Discussion |
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<td>12.30</td>
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<td>• On track for 2020? Towards the WHO roadmap's targets for neglected</td>
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<td>tropical diseases - Echinococcosis control: Bernadette Abela-Ridder /</td>
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<td>Elkhan Gasimov (WHO, Switzerland) - 30 min</td>
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<td>• An integrated approach to the control of Echinococcosis: the South</td>
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<td>American Initiative: Pilar Irabedra (Uruguay) - 30 min</td>
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<td>• Surveillance of human echinococcosis in EU/EEA – from trends to</td>
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<td>perceived EU priorities: Johanna Takkenen (ECDC, Sweden) - 30 min</td>
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<td>• Carmen-Michaela Gretu - President of the Congress</td>
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HERACLES collaborative project on cystic echinococcosis funded by the European Commission (FP7)

Adriano Casulli*, Bernadette Abela-Ridder†, on behalf of HERACLES consortium‡

1. European Union Reference Laboratory for Parasites (EURILAB); Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy; 2. Mar Siles-Lucas (Parasitology Unit, Instituto de Recursos Naturales y Agrobiologia de Salamanca, CSIC, Salamanca, Spain); Michaela Carmen Cresta (“C. Davila” University of Medicine and Pharmacy, Coletina Clinical Hospital – Parasitology, Bucharest, Romania); Kamenata Vutova (Specialised Hospital of Infectious and Parasitic Diseases “Prof. Ivan Kirov”, Department of Infectious, Tropical and Parasitic Diseases, Medical University – Sofia, Bulgaria); Olan Akkan (Department of Radiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey); Gulay Vural (Department of Parasitology, Faculty of Veterinary Science, Namik Kemal University, Tekirdag, Turkey); Aranzta Cortés (Vireill, SL, Granada, Spain); Michela Renzulli (ALTA S.r.l., Siena, Italy); Enrico Brunetti and Francesca Tammarozi (Department of Clinical, Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Italy; WHO Collaborating Centre for the Clinical Management of Cystic Echinococcosis); 3. Department of Control of Neglected Tropical Diseases, World Health Organization (WHO), Geneva, Switzerland.

*Presenting author. E-mail: adriano.casulli@iss.it

Aims: Cystic Echinococcosis (CE) is one of the most important zoonotic diseases and was recently assigned to the WHO list of neglected tropical diseases. Clinical decision making on CE is difficult because the evidence base is low as no funding to support randomized clinical trials is available. HERACLES (Human cystic Echinococcosis ResArch in CentrAL and Eastern Societies) is an EU funded collaborative project that offers for the first time a reasonable amount of funding and a real chance to break this vicious circle and finally put CE on the radar.

Materials and methods: The main goals of the HERACLES cooperative project are to: identify the population affected by CE in Eastern Europe by extended ultrasound screening; create the European Register of CE (ERCE); establish the Echino-BioBank to extend standardized investigations on CE for researchers and clinicians; set-up and validate new molecular-based PoC-LoC (Point of Care - Lab on a Chip) kits based on recombinant antigens; identify cyst stage-specific biomarkers through “omic” studies; increase drug bioavailability of benzimidazoles; train experts working in Eastern European countries, as they are crucial to fight this disease.

Results: In December 2015 we built ERCE (http://www.heracles-fp7.eu/erce.html) with 656 patients enrolled as of this writing and under the umbrella of the Heracles project an ultrasound screening of 12,050 people was carried out in summer 2014 in Bulgaria, Romania and Turkey. A total of 24,000 people are expected to be screened by the end of 2015. A Biobank for samples (EchinoBiobank) hosting >900 samples to date and a retrospective database for clinical data (CYSTRACK) have also been developed.

Conclusions: The results from HERACLES will support government, European Commission, related European agencies (ECDC and EFSA), international organizations (WHO) and the Global Burden of Disease study (IHME) to harmonize data collection, monitoring and reporting of CE. We see this as a breakthrough in the current scenario of CE and we want to seize this opportunity by adding multipliers to the already broad field of action of HERACLES, by involving more partners and adding activities under the umbrella of HERACLES “Extended Family” (http://www.herokuapp-fp7.eu/interactive_map.html).

Acknowledgements: The research was funded from the European Community’s FP7 under the grant agreement 602051 (Project HERACLES; http://www.Heracles-fp7.eu/).

Experience from HERACLES Project: ultrasound screening for CE in Bulgaria in 2014

K. Vutova*, B. Golemanov‡, R. Chipova‡, E. Aleksandrova‡, V. Krashev‡, M. Muhtarov, F. Tamarozzi‡, E. Brunetti‡, A. Casulli‡, S. Badalanov‡

1. SBALIPB “Prof. Ivan Kirov” Sofia, Department of Infectious Diseases, Parasitology and Tropical Medicine, Medical University – Sofia, Bulgaria; 2. University Hospital “Queen Joanna”, Department of Gastroenterology, Medical University – Sofia, Bulgaria; 3. Hospital “Kardzhali”, Gastroenterology ward, Kardzhali town, Bulgaria; 4. Department of Clinical-Surgical, Diagnostic and Paediatric Sciences, WHO Collaborating Centre for Clinical Management of Cystic Echinococcosis, University of Pavia, Italy; 5. European Union Reference Laboratory for Parasites, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy.

* Presenting author. E-mail: k_vutova@abv.bg

Aims: Human cystic echinococcosis (CE) is a chronic disease in humans which is usually asymptomatic, but in some cases is very severe and with fatal outcome. This parasitosis is a major health and economic problem in many areas in the world. High incidence of CE reported in Eastern Europe was a reason to support HERACLES international project funded by the Seventh Framework Programme (FP7) of the European Commission.

Materials and Methods: A study of prevalence of abdominal CE was undertaken in Bulgaria and conducted by the Hospital of Infectious and Parasitic Diseases “Prof. J. Kirov” (Sofia). A first year ultrasound (US) screening was performed on 5,332 people during the summer and winter period in 2014. US was conducted in two Bulgarian endemic regions for CE, such as Kardzhali and Blagoevgrad districts. Ethical approval was given by Ethical Committee from “Prof. J. Kirov” Hospital. Informed consent was obtained from participants and a questionnaire reporting demographic and epidemiological information was distributed. Each suspected case was examined independently by 2 clinicians and patients were assigned to treatment according to WHO-Informal Working Group on Echinococcosis (WHO-IWGE) Expert Consensus and the study protocol.

Results: US evaluation in the abdomens for CE and other various diseases was performed. Total number of patients evaluated with cyst lesions was 45, of which 16 were identified with CL stage (WHO-IWGE classification). Hydatid cysts were found in 26 patients, of which 10 were post-operated cysts without relapse (CE4), 1 patient received PAIR and 4 were treated with albendazole in the past (solid lesions, CE4 / CE5). In 10 patients newly hydatid cysts were found (CE2 and CE4) and recurrences were detected in 3 patients operated in the past.

Conclusion: Extended US screening in high endemic areas for CE was carried out for the first time in Bulgaria and was supported with great interest by local populations. Collection and analysis of accurate epidemiological and clinical data will give a reliable picture of the burden of this disease in Bulgaria, providing a statistically supported case series for future evaluation of efficacy and effectiveness of interventions.

Acknowledgements: The research was funded from the European Community’s FP7 under the grant agreement 602051 (Project HERACLES).
Experience from HERACLES Project: ultrasound screening for CE in Turkey in 2014

Okan Akhan1*, Devrim Akinci1, Turken Gıftci1, Burcu Akpinar1, Francesca Tamarozzi2, Enrico Brunetti3, Adriano Casulli4, Serra Orsten1
1. Hacettepe University, Faculty of Medicine, Department of Radiology, Ankara, Turkey; 2. European Union Reference Laboratory for Parasites, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy; 3. Hacettepe University, Faculty of Medicine, Department of Medical Microbiology, Ankara, Turkey; 4. Department of Clinical-Surgical, Diagnostic and Paediatric Sciences, WHO Collaborating Centre for Clinical Management of Cystic Echinococcosis, University of Pavia, Italy

* Presenting author e-mail: akhan@tr.net

Aims: Among the clinical forms of the human disease, cystic echinococcosis (CE) is due to Echinococcus granulosus complex, a globally-distributed parasitic disease and a public health threat at global level, including Turkey. In this study, prevalence of abdominal CE was investigated in regions where agricultural and animal breeding activities are frequent and previous information on the disease is limited. This research was conceived under HERACLES project, 7th Framework Programme of the European Commission.

Material and methods: Four regions with different geographical and epidemiological properties, which include Cubuk district of Ankara province (Central Anatolia), Uzunkopru district of Edirne province (Eastern Thrace), Akcakale district of Sanliurfa province (Southeastern Anatolia) and Tatvan district of Bitlis province (Eastern Anatolia) were examined. The presence of CE was investigated via ultrasonography (US). Ethical approval was given by Ethical Committee from the Faculty of Medicine of Hacettepe University. Informed consent was obtained from participants and a questionnaire reporting demographic and epidemiological information was distributed. Each suspected case was examined independently by 2 clinicians and patients were assigned to treatment according to WHO-Informal Working Group on Echinococcosis (WHO-IWGE) Expert Consensus.

Results: Among the 3465 individuals examined, a total of 24 CE cases were identified. The raw prevalence of CE cases by region was: 1.17% in Akcakale, 0.82% in Uzunkopru, 0.53% in Cubuk and 0.12% in Tatvan. Echinococcal cysts localization was as follow: 89.6% in the liver, 6.8% in the kidney and 3.6% in the lung. Five individuals (17.2%) had two CE cysts. The size of the cysts was between 1.23 and 13.94 cm.

Conclusion: CE cases have been detected in all regions studied. Follow-up, laboratory testing and treatment of the current cases are underway. Collection and analysis of accurate epidemiological and clinical data will give a reliable picture of the burden of this disease in Turkey.

Acknowledgements: The research that led to these results has received funding from the FP7 (HERACLES) g.a. 602051.

Experience from HERACLES Project: ultrasound screening for CE in Romania during 2014-2015

Carmen-Michaela Creţu1,2, Cosmin Popa1,2, Gabriela Loredana Popa1,2, Patricia Mihailescu1, Cerasella Dragomirescu1, Mirea Ioan Popa1, Denisa Janta1, Corina Constantin1, Marius Petretescu1, Francesca Tamarozzi2, Enrico Brunetti3, Adriano Casulli4
1. “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania; 2. Colentina Clinical Hospital, Bucharest, Romania; 3. Eco-Para-Diagnostic SRL, Bucharest, Romania; 4. National Institute of Public Health, Bucharest, Romania; 5. University of Pavia, Italy; 6. European Union Reference Laboratory for Parasites, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

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Aims: Cystic echinococcosis (CE), a neglected disease caused by the larval stage of Echinococcus granulosus complex, is widely distributed all over the world. Romania is a highly endemic territory, representing a public health priority. The present US survey was ruled out during 2014-2015, under the framework of FP7-HERACLES Project, in rural areas of Romania, in order to estimate the real prevalence of abdominal CE and to identify the risk factors.

Material and methods: Two counties were selected in 2014 (Giurgiu and Brăila) and three in 2015 (Neamț, Argeș, Vaslui), based on the official reported data on CE and the provenience of the patients in Colentina Clinical Hospital (Bucharest, Romania). After the dissemination process focusing on the disease, preventive measures and purposes of the survey, the informed consent was signed, the questionnaires for demographic and epidemiological data were filled in and the screening started. Each suspect/positive case was carefully analyzed and allocated to treatment according to WHO – Informal Working Group on Echinococcosis (WHO-IWGE) – Expert consensus and to the study protocol.

Results: A total number of 7,469 people, living in 26 villages and aged between 3-90 years old, were screened (2,902/2014 and 4,567/2015). Positive or suspect cases for CE were 127 (65/2014 - 2.23% and 62/2015 - 1.42%). Higher number of females were enrolled in the trial (1,978/2014 - 68.15% and 3,135/2015 - 68.64%) with a discovery rate of CE cases of 1.73%/2014 and 1.43%/2015 for female and 2.47%/2014 and 1.18%/2015 for male. All cyst stages (CE1-CE5) were found. Co-morbidities were noticed in 39.9% in 2014 and 57.4% in 2015 (liver steatosis, tumors, gall bladder stones, kidney stones, uterus fibroma etc).

Conclusions: Cases of CE were found in all villages where the screening was carried out. Collection and analysis of accurate epidemiological and clinical data will give a reliable picture of the burden of this disease in Romania, providing a statistically supported case series for future evaluation of efficacy and effectiveness of interventions. Biological samples collected during the surveys (positives and controls) will support HERACLES BioBank. Implementation of a sustainable National Control Program seems to be necessary.

Acknowledgements: The research was funded from the European Community’s FP7 under the grant agreement 602051 (Project HERACLES).
Aims: Cystic Echinococcosis (CE) is highly endemic in some areas of Europe, but its true burden is unknown due to the lack of efficient, specific and mandatory reporting systems. Neglect hampers the collection of good quality data to inform evidence-based diagnostic and therapeutic strategies, adding to the lack of prospective randomized trials and resulting in suboptimal, when not wrong, management of cases outside referral centres and ineffective allocation of public resources. In the context of HERACLES project, the European Register of Cystic Echinococcosis (ERCE; www.heracles-fp7.eu/erce), was launched in October 2014, by expanding the Italian prospective register of CE patients (RIEC).

Material and methods: ERCE is a prospective, observational, multicentre register of patients with probable or confirmed CE (according to WHO-IWGE classification) enrolled in hospital, outpatient setting or during HERACLES ultrasound surveys. Each patient is assigned a unique identification code, allowing movement from one centre to another with no data loss or duplication. ERCE is structured taking into account the peculiar features of CE, epidemiological, diagnostic and clinical data are recorded for each follow-up.

Results: As of July 2015, 28 centres adhered to ERCE: 21 from Italy, one centre from Bulgaria, Albania, Austria, Poland, Romania, Turkey and UK. A total of 656 patients are at the moment enrolled in ERCE. In Bulgaria, Romania and Turkey, during 2014 HERACLES ultrasound surveys for CE case detection, more than 100 patients were enrolled. Centers from Albania, Austria and Poland are currently part of the ERCE “extended family” (http://www.heracles-fp7.eu/interactive_map.html), while France, Greece, Hungary and The Netherlands are going to join the register, after the approval of their Ethical Committee.

Conclusions: ERCE responds to a long standing need for a CE Register with online data entry and recorded data largely outnumber the total of National cases reported by most European endemic countries. This confirms the need for a better report system of CE at European level. Thus, to encourage new centres to join and to ensure regular data entry in ERCE, a major effort was made to improve the structure and usability of the database and to implement “benefits” for the participating Centres.

Acknowledgements: The research was funded from the European Community’s FP7 under the grant agreement 602051 (Project HERACLES; http://www.heraclces-fp7.eu/).

Surveillance of human echinococcosis in EU/EEA – from trends to perceived priorities

Johanna Takkinen
Head of Food- and Waterborne Diseases and Zoonoses programme, Office of Chief Scientist, European Centre for Disease Prevention and Control (ECDC)
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European Centre for Disease Prevention and Control (ECDC) has a mandate to identify, assess and communicate threats to human health from communicable diseases(1). As part of the ECDC’s mandate, the European Surveillance System (TESSy) has been established in 2008 for systematic collection, validation, analysis and dissemination of data on specified infectious diseases. The system covers today surveillance of 52 communicable diseases and special public health issues, like antimicrobial resistance. Member States in the European Union (EU) and European Economic Area (EEA) report predefined data for each disease depending on the availability of data in their own country and according to the EU case definitions(2).

Echinococcosis is one of the five parasitic foodborne diseases that is covered by EU-wide surveillance under the ECDC Food- and Waterborne Diseases and Zoonoses programme. Twenty-four countries have mandatory notification of echinococcosis cases, four have voluntary system and three countries don't report cases to TESSy (Denmark, Italy and Liechtenstein). The EU case definition defines five diagnostic criteria of which at least one has to be fulfilled for reporting of a confirmed echinococcosis case: 1) Histopathology or parasitology compatible with Echinococcus multilocularis or granulosus (e.g. direct visualisation of the protoscolex in cyst fluid), 2) Detection of Echinococcus granulosus pathognomonic macroscopic morphology of cyst(s) in surgical specimens, 3) Typical organ lesions detected by imaging techniques (e.g. computerised tomography, sonography, MRI) AND confirmed by a serological test, 4) Echinococcus spp. specific serum antibodies by high-sensitivity serological test AND confirmed by a high specificity serological test, and/or 5) Detection of Echinococcus multilocularis or granulosus nucleic acid in a clinical specimen(2).

The reporting of species level information is optional and many countries don’t differentiate the causative parasite as E. granulosus or E. multilocularis. This remains a challenge and a place for improvement as the clinical disease and treatment options vary markedly between the two species.
Between 2009 and 2013, a stable trend of 0.18 cases per 100 000 population has been noted. In 2013, 794 confirmed cases were reported by 28 countries. Fifteen countries provided species information. Six countries (Bulgaria, Latvia, Netherlands, Norway, Portugal, Slovenia and United Kingdom) only reported cases of E. granulosus, two countries (Austria and France) only reported E. multilocularis cases, and six countries (Austria, Belgium, Germany, Lithuania, Poland and Slovakia) reported both parasites in humans\(^\text{1}\). Of 543 echinococcosis cases with known data of species, 79\% were E. granulosus and 21\% E. multilocularis. Two deaths were reported in 2013, one from Austria and one from Germany, both due to E. multilocularis\(^\text{2}\). While the overall reporting of echinococcosis cases has been stable in EU, the trend by species is difficult to ascertain due to highly variable laboratory practices and reporting routines to EU level.

In general, the diagnosis and reporting of food-borne parasites is largely underestimated\(^\text{3,4}\). In order to assess the underestimation in EU/EEA and to explore the perceived burden and importance of the five foodborne parasitic infections under EU surveillance (toxoplasmosis, echinococcosis, giardiasis, cryptosporidiosis and trichinellosis) and the 24 foodborne parasites globally ranked by UN-FAO in 2014\(^\text{5}\), ECDC launched a survey for Member States in 2014. In the survey, echinococcosis was divided into cystic and alveolar forms. For the global ranking exercise, countries were asked to rank the listed FAO/WHO foodborne parasites in a priority order from their country perspective by scoring the pre-defined factors affecting under-ascertainment, under-reporting and burden. In the ranking of 24 foodborne parasites, E. granulosus ranked the second highest after toxoplasmosis whereas E. multilocularis was on the sixth place although there were some variations by regions. For example, the Western Countries gave higher priority for E. multilocularis compared to E. granulosus whereas Southern and Eastern Regions gave a reverse priority order. Regarding the factors related to the burden, the three most important mentioned for E. granulosus were: 1) national prevention and control measures, 2) morbidity and 3) economic impact to the individual. For E. multilocularis, the respective three factors were slightly different: 1) national prevention and control measures, 2) economic impact to the individual and 3) EU level prevention and control measures. The likelihood of an increase in case numbers was considered relatively high for E. multilocularis but low for E. granulosus. The most important perceived factor affecting underreporting of echinococcosis was related to reporting by clinicians, both from private and public sector. The three main reasons for under-ascertainment of echinococcosis cases were: 1) asymptomatic infections, 2) lack of awareness by clinicians and 3) inadequate diagnosis. The most important barriers for reporting to EU level were lack of staff followed by lack of mandatory reporting and regional differences in data collection.

Surveillance of echinococcosis at EU level is heavily relying on the case ascertainment and reporting systems in the countries. Our survey in the EU and EEA countries provides valuable information on factors affecting ascertainment and reporting as well as the perceived disease burden. This will help ECDC to plan targeted actions on those factors that can markedly improve surveillance.

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References:
The use of the 2B2t recombinant antigen in a point-of-care diagnostic tool for the serodiagnosis of CE patients: preliminary results in immunoStrips

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Aims: Human cystic echinococcosis (CE) is usually diagnosed by imaging techniques. Serology against the native hydatid fluid (HF) is used as a confirmatory test. However, this test has some drawbacks, such as the lack of specificity and sensitivity, and the difficulties in the standardization of a native antigen. With the aim of improving serology tests for CE diagnosis, a new immunochromatographic test (ICT) prototype using the 2B2t recombinant antigen has been developed and evaluated under the umbrella of the HERACLES project.

Materials and methods: The first prototype carried the 2B2t both in the conjugate (C) and the test line (TL). The second prototype was prepared with semi purified HF in the C and the 2B2t in the TL. This was further improved by adding a second reactive in the C, either protein G or a human anti-IgG4. The final prototype containing semi purified HF plus anti-IgG4 in the C and the 2B2t in the TL was tested against 36 sera from healthy donors, 50 sera from alveolar echinococcosis patients and 371 sera from 190 CE patients. The reactivity of the prototype was tested after storage of the ICT at 50°C for one month to check its stability.

Results: The 2B2t in the C and the TL was less sensitive and specific than the original ICT carrying HF in both C and TL. When the 2B2t was used only in the TL combined with HF in the C or with HF plus protein G in the C, sensitivity was still lower than that displayed by the ICT containing only HF. When HF and a human anti-IgG4 antibody were combined in the C, together with the 2B2t in the TL, the prototype showed similar sensitivity for CE patients with active and transitional cysts (96%) and higher specificity (81% vs. 58%) than the ICT containing only HF. The sensitivity of the prototype and the ICT containing only HF were both statistically influenced by the number, size and stage of cysts. The reactivity of the prototype was the same before and after storage at 50°C for one month.

Conclusion: The 2B2t recombinant antigen shows equal sensitivity and better specificity than the HF when used in the TL of the ICT. Nevertheless, the 2B2t should be combined with HF in the C to reach the optimal reactivity. Studies combining several recombinant antigens both in the TL and the C to fully substitute the native antigen in the ICT test have been planned.

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EchinoBiobank: a new tool for standardizing research on cystic echinococcosis

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Aims: Creating an Echinococcus biobank is one of the deliverables in the HERACLES project (http://www.heracles-fp7.eu/). Samples from patients and animals will be hosted in Salamanca, Spain (IRNASA, CSIC) after transfer agreement.

Material and methods: In March 2015 the EchinoBiobank was officially registered. By August 2015, the biobank has received samples (serum, buffy coat, plasma and plasma DMSO) from Ankara, Turkey (18 CE patients and 50 donors), and from Pavia, Italy (603 sera of 253 CE patients). Additionally, the biobank hosts 81 samples of cysts collected in 13 pigs, 8 sheep and 6 cows from different Spanish slaughterhouses and six recombinant antigens (Ag5, AgB1t, Ag2B2t, AFFP, MDH and CaBP) produced in the IRNASA. The biobank will receive samples from surveys in Bulgaria and Romania. All samples are anonymized and 2D codified to ensure data accuracy and traceability.

Results: Italian serum samples have been used for the preliminary validation of the above mentioned recombinant antigens. The results show their potential use as markers for cyst stage detection but this needs further validation with an extended number of samples (results presented by Sánchez-Ovejero et al.). Vircell (http://www.vircell.com/) has started the validation of those antigens in ImmunoStrips with sera provided by the EchinoBiobank (results presented by Delgado et al.). Animal cysts have been used for preliminary studies on the presence and cargo composition of exosomes in hydatid fluid (results presented by Manzano-Román et al.). The recombinant Ag5 and CE sera will be transferred to Porto Conte Ricerche (http://www.portocontericerche.it/en/) for its validation in ELISA and comparison with the native Ag5.

Conclusions: The EchinoBiobank is a crucial tool for future activities inside HERACLES. Additionally, it will serve to extend standardized investigations on CE for researchers and clinicians, focusing on aspects such as parasite biology, host-parasite interplay, parasite genotyping, patients’ clinical management standardization etc. This is the most appropriate tool to produce evidence-based conclusions in the field of CE and, as such, is invaluable and unique. Since this tool is available to every specialist working on CE, to broaden the number of samples hosted in this biobank through donations from different institutions will be highly beneficial for the advance in the field of CE.

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Serology for the diagnosis and follow-up of CE: is it worth using it?

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Aims: Cystic echinococcosis (CE) is a chronic disease in which prognosis depends on multiple factors, including location, number, size and stage of the cysts, making CE a disease of complex clinical management. Only experts’ recommendations are available but no evidence-based conclusions have been drawn for CE clinical management. One of those pitfalls refers to the lack of evidence to support the use of the currently available serological tools for the diagnosis and follow-up of CE patients. In this respect, crude antigens from different cyst stages (results presented by Delgado et al.) are used to detect specific antibodies in patients, giving rise to false positive and negative results. In an attempt to overcome the drawbacks on the use of HCF for the serology of CE patients, several purified fractions of HCF, mainly containing Ag5 and AgB, have been generated and tested by several authors. Additionally, the advent of molecular techniques allowing the production of recombinant proteins as candidates antigens that could overcome the problems associated with the use of crude parasite extracts. Nevertheless, testing of these new molecules has shown very variable results among different laboratories, probably due to the lack of a systematic approach for their validation. In this respect, the Echinobiobank created under the HERACLES umbrella (http://www.heracles-fp7.eu/) supports these activities with the repository collection of serum samples and associated clinical data.

Methods and results: The studies on the performance of new antigens and the crude HCF for the detection of specific antibodies/isotypes/subisotypes, together with proteomic approaches applied at different cyst stages, show that the composition of the HCF could be influenced by the stage of development of the cysts and the parasite genotype. Additionally, factors like number and size of the cysts could influence the production of antibodies in a qualitative and quantitative manner. These variables could be used to propose the detection of different antibodies against defined parasite antigens not only to confirm the disease, but also to differentiate among distinct clinical stages of CE.

Conclusion: An overview on usefulness of the serological tools currently available for the diagnosis and follow-up of CE patients will be given. Additionally, new data on antigen composition and molecular developments will be presented.

Acknowledgements: This research has received funding from the FP7 (HERACLES) g.a. 602051.
Cystic echinococcosis (CE) is a chronic disease and early diagnosis of CE in humans is difficult because of asymptomatic features in the early stages of infection and for a long time after establishment of the parasite. Actually, the diagnosis of CE is currently based on imaging techniques, such as ultrasonography or chest X-ray, computed axial tomography and magnetic resonance imaging. Sometimes it’s difficult to detect the newly growing or atypical cysts with imaging techniques. Therefore, immunological tests have been considered to be useful confirming imaging techniques and also clinical findings. Serology of CE has a very old history. Almost all serological tests, such as Casoni intradermal test, complement fixation test, indirect hemagglutination or latex agglutination, immunoelectrophoresis, indirect fluorescent antibody test, ELISA and immunoblotting tests have been used in the diagnosis of human CE. Actually, the main problems in the immunodiagnosis of CE are the often unsatisfactory performances of the tests and the difficulties on the standardization of antigen preparations and techniques. Therefore, the results of serological tests depend on multiple factors, such as antigen quality, test system, organ site and number of hydatid cysts, individual variability of immune responses etc. Hydatid cyst fluid has been used as a main antigenic source for the primary immunodiagnosis of human CE and also for the follow up of patients after treatment (surgical or pharmacological) until now. But there are some inconveniences, such as cross-reactivity with other helminthes (mainly E. multilocularis, T. solium etc.). Because of this, even if HCF is considered the main antigenic source for the immunodiagnosis of human CE, it has now become more frequent to purify components such as the lipoproteins antigen B and antigen 5, the most relevant components of HCF for diagnostic purposes. Immunoblot detection of the smallest subunit (8 kDa) of AgB has proved the most reliable tool in diagnostic studies. It has been suggested that using recombinants proteins or by combining well-defined antigens, including synthetic peptides, characterization of new antigens and development of recombinant antigen-based assays improve the performance of CE immunodiagnosis. However, there is still no standard, highly sensitive and specific serological test for antibody detection in cases of human CE until now. Recently molecular tools have been used not only for evaluation of viability of the cyst but also for getting a better understanding of the E. granulosus genome and gene expression/regulation which is essential for developing new treatments. Strain identification is also important establishing control programs of CE.

**Repurposed drugs and novel drug targets for the treatment of echinococcosis**

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The current chemotherapy of alveolar (AE) and cystic echinococcosis (CE) is based on benzimidazoles and has surely contributed to a significant improvement of living conditions and increased the life span of affected patients. However, treatment failures and the occurrence of side effects have been reported. Thus, there is an inherent interest in developing novel and improved chemotherapeutical options. The model that is used in our institute is *Echinococcus multilocularis*. *E. multilocularis* metacestodes proliferate asexually by exogenous and/or endogenous budding. This process can be mimicked *in vitro* and thus renders this model highly suitable for screening of anti-parasitic drugs. One approach to speed up drug discovery for AE is “drug repurposing”, e.g. to find new uses for already approved drugs or compounds / compound classes that are already on the market or in development for other indications.

A range of *E. multilocularis* cell- and metacestode- based drug screening assays were developed in our laboratory, which can be used for monitoring of drug-induced physical alterations in metacestodes and for impairment of parasite cell viability. We characterized mefloquine-, artemisinin- and pentamidine- derivatives, ruthenium-based organometallic compounds and other benzimidazoles with respect to their capacities to kill *E. multilocularis* cells and metacestodes *in vitro*. The mouse model is being used for *in vivo* assessments of the efficacy of compounds with outstanding *in vitro* properties and has been further optimized, standardized and refined. In addition, novel formulations including micro- and nanocarriers are being used to optimize drug exposure and improve the efficacy of interesting compounds. We further exploited *in vitro* assays to demonstrate the efficacy of drugs derived from compound libraries from different commercial and non-commercial sources (FDA, MMV-malaria box, several academic collaborators), and this has allowed us to identify novel drug targets in *E. multilocularis*, which can now be further investigated and validated. In addition, investigations on potential targets of selected drugs in *Echinococcus* will shed light on their functions and these insights can be useful to explore their potential mechanism of action in the metacestode stage.
Watch and wait for cystic echinococcosis

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There are four management options for CE: surgery, percutaneous treatment, medical treatment with benzimidazoles and watch and wait or expectant management. Expert clinicians in some referral centers have long managed expectantly inactive cysts of the liver that remain free of complications for years. Our group has recently shown in detail for the first time that this option is viable in referral centers when strict follow-up can be implemented and patients are compliant. If the safety of this approach is confirmed on larger cohorts, this simple, inexpensive option can save an enormous amount of useless drug prescriptions and surgical interventions, as a remarkable percentage of patients with hepatic CE harbor inactive, non-complicated cysts. Although CE2 and CE3b cysts are probably best treated with surgery (there are no studies comparing these two treatments) in selected (i.e. uncomplicated) echinococcal cysts of the liver, watch and wait can also be applied as a bridge to surgery, but this needs further study.

Controversies in the clinical management of cystic echinococcosis. Is it time for a change?

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In 2010 the World Health Organization Informal Working Group on Echinococcosis published updated consensus guidelines for the management of human echinococcosis. Five years later an international survey demonstrated wide variation in the management of cystic echinococcosis (CE) worldwide. Non-adherence to the consensus guidelines was common, especially in respect of recommended duration of post-operative albendazole therapy. Praziquantel use also varied greatly. Furthermore, WHO-IWGE guidance suggests a dose of 40 mg/kg weekly in addition to daily albendazole, but cites a reference describing the effect of a pre-operative course using praziquantel 25 mg/kg daily, plus daily albendazole.

A change of approach is needed. The WHO-IWGE guidelines need to be revised and, in some areas, made more prescriptive. The recommendation for use of praziquantel needs to be changed. The balance between the duration of pre- and post-procedure albendazole therapy needs to be re-evaluated and matched more closely to cyst type.

References
Cystic echinococcosis (CE) is caused by a diverse array of *Echinococcus* species and intra-specific variants. A high diversity of CE agents in Europe, western Asia and South America contrasts with e.g. East Asia and Australia, where *E. granulosus* s.s. is the only or the dominating taxon. In Africa, the spread and impact of different CE agents was until recently largely unknown, with the notable exception of the Maghreb and a focus in eastern Africa. Starting in 2009, the ongoing collaborative project CESSARI, involving research institutions from Sudan, Ethiopia, Kenya, Uganda, Zambia, Namibia, South Africa and Germany, has gradually been shedding light on the complex epidemiological situation. All described species and major genotypes causing CE (with the exception of the “cervid strains”) are present in sub-Saharan Africa, one species (*E. felidis*) and an as yet undescribed divergent variant of the *E. granulosus* s.s. cluster are probably endemic for the continent. Distribution and frequency of the various taxa are highly uneven between and within countries, and some taxa use different domestic and wild host animals in different regions. Major project results can be summarized as follows:

**E. granulosus s.s.**: Present in all surveyed countries except Zambia, but frequency differs. Largely absent in (northern) Sudan, probably rare in Namibia, particularly frequent in parts of East Africa. Occurrence of this species correlates with high prevalence of human disease. Very wide host range, but transmission usually via sheep.

**E. canadensis** G6/7: Dominant in most of Sudan, frequent in northern East Africa, rare or sporadic elsewhere. High prevalence in livestock (camels) is not reflected in high numbers of human cases (except in risk foci). Wide host range, transmission in northeastern Africa is usually based on camels. Widespread in wildlife of southern, but not eastern Africa.

**E. ortleppi**: Widespread, but sporadic in most regions, more frequent in some traditional cattle-husbandry areas (e.g. western Zambia). Human cases are known, but rare.

**E. equinus**: South of the Sahara, this species was so far only molecularly confirmed in lions, jackals and zebras in southwestern Africa. Probably non-pathogenic to humans.

**E. felidis**: Wildlife parasite involving lions, hyenas and warthogs in eastern and southern Africa. Frequent in conservation areas, but not yet found in humans or any domestic animal.

### Aim:
Cystic echinococcosis (CE) is one of the most important zoonotic diseases in sub-Saharan Africa especially in the rural pastoralist communities. CESSARI, a research initiative on cystic echinococcosis in sub-Saharan Africa, is a multi-country and multi-institutional project whose aim is to characterize the epidemiological and clinical implications of the newly established diversity of *Echinococcus* forms causing CE and to fill gaps of knowledge within the African countries covered by this consortium.

### Materials and methods:
Human, livestock, dog and wild life surveys were carried out in various countries and samples obtained analyzed through microscopy, PCR-RFLP and sequencing for species and genotype identification.

### Results:
The prevailing *Echinococcus* species/strain is likely to be crucial for the impact on public health. In Sudan, human CE is more frequent than previously expected with high prevalence existing in certain foci and associated with risk behaviour. Livestock CE is generally frequent, especially in camels. The highly pathogenic *E. granulosus* sensu stricto is frequent in Southern Sudan, causing 2.5% prevalence of human CE, but there seems to be limited northward spread. The first ultrasound surveys since 30 years in Ethiopia revealed high prevalence of human CE in southern Ethiopia. In Kenya, the geographical endemicity pattern shows two high-prevalence foci of human and livestock CE (northwest and south-southwest), with low endemicity regions in-between. In the foci, the frequency of human CE is stable and high, the frequency of livestock CE is regionally even increasing.

The first country-wide ultrasound surveys for human CE in Uganda reveal high prevalences in all parts of the country, while in Zambia human and livestock CE are moderately frequent in the western part of the country. To date, only the cattle-associated *E. ortleppi* was identified in Zambia.

In South Africa, hospital data indicate a far larger public health impact of CE than previously assumed. There is high diversity and geographical spread of *Echinococcus* spp. in numerous species of wildlife. Exclusive wildlife cycles were identified in Kenya (*E. felidis*), Uganda (*E. felidis* and Namibia (*E. equinus*, *E. felidis*).

### Conclusion:
The epidemiological situation of CE and its causative agents is highly diverse between and within countries of Sub-Saharan Africa.

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**Species, genotypes and life cycles: news out of Africa**

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The taxonomic revision of *Echinococcus granulosus* sensu lato has been very topical for the past three decades. While the species status of *E. granulosus* sensu stricto (G1 – G3), *E. equinus* and *E. ortleppi* is generally accepted, the *E. canadensis* (G6 – G10) cluster is still under dispute as oversimplified, demanding more genetic and ecological data for resolution. Here, we present the haplotype structure of *E. canadensis* G6/7 and *E. ortleppi* based on complete cox 1 (1608 bp), nad 1 (894 bp), and cox 1/nad 1 concatenated DNA (2502 bp) sequences mainly from sub-Saharan Africa, supplemented by published data (GeneBank). Hydatid cyst and adult worm materials from livestock, dogs and humans were obtained from Kenya (79 E. canadensis and 43 E. ortleppi), Ethiopia (1 E. canadensis and 7 E. ortleppi), Sudan (55 E. canadensis and 6 E. ortleppi), Zambia (53 E. ortleppi) and Namibia (1 E. ortleppi), and in addition non-African isolates from Brazil (79 E. ortleppi), France (3 E. canadensis and 3 E. ortleppi) and Slovakia (7 E. canadensis and 2 E. ortleppi). In total, we found 18 cox 1 haplotypes, 15 nad 1 haplotypes and 30 cox 1/nad 1 (concatenated) haplotypes in the three countries. Based on concatenated sequences, Kenya and Sudan shared only two haplotypes in livestock, while twenty eight haplotypes were unique for the countries (1 from Ethiopian cattle, 14 from Kenyan livestock, dogs and humans and 13 from Sudanese livestock and humans). Host preference was not apparent for any haplotype, but the largest proportion of the isolates came from camels. Haplotype diversity was similar in Kenya and Sudan, but only few haplotypes were shared, indicating limited exchange of livestock despite the proximity. None of the countries shared any haplotype with the non-African countries. In contrast to *E. canadensis* G6/7, all *E. ortleppi* isolates (African and non-African) showed minimal variance in the concatenated cox 1/nad 1 sequences. Four haplotypes were found only in Zambia, two were widespread in Zambia, Kenya, Namibia and Ethiopia, two only occurred in Sudan, two in Ethiopia, one in France and one in Brazil. Tajima’s D and Fu’s Fs were positive for *E. ortleppi* from Kenya, Sudan and Zambia except in Ethiopia where Tajima’s D = -1.358 and Fu’s Fs = -0.237 indicating parasite’s population expansion. *E. ortleppi* population in the other countries might have suffered recent bottlenecks. The present data provide the background for further biogeographical and taxonomic studies on these parasites.

**Cystic echinococcosis in a central African tropical rainforest ecosystem – an Epidemiologic pilot study from Gabon**

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**Aim:** Cystic echinococcosis (CE) is thought to have an almost worldwide distribution. CE is listed as occurring in most regions of sub-Saharan Africa with varying prevalence, but epidemiological data for Central Africa are to date virtually non-existing. We therefore conducted an epidemiologic field survey in central Gabon – a rural Central African tropical ecosystem.

**Methods:** Volunteers were informed about the project and, after informed consent was given, abdominal ultrasound examination was performed and blood was drawn for serologic analyses. A questionnaire on risk factors was completed. For serologic analysis, a commercial indirect haemagglutination assay was used (CelloGnost Echinococcosis, Siemens) as screening test and if positive, a Western Blot was performed (Echinococcus WB, LDBIO Diagnostics) as confirmatory testing. In addition, canine stool samples were collected for microscopic and PCR-analysis.

**Results:** In total, 347 patients were screened by ultrasound and serologic analyses for cystic echinococcosis. 198 (57.1%) were female and 149 (42.9%) were male. Patients from 19 different communities in the province of Moyen-Ogooue were included. No cystic lesions compatible with cystic echinococcosis were detected in ultrasound screening. Nine volunteers (2.6%) where positive for echinococcosis in the IHA screening test. None was positive in the Western Blot. 46 canine stool samples were analysed microscopically and via PCR. None of the samples was positive for echinococcosis.

**Conclusion:** This epidemiologic pilot study provides preliminary epidemiologic data for CE in a Central African ecosystem. No suspected cases of CE could be identified and none of the collected canine stool samples contained DNA of *Echinococcus granulosus*. Although sample size was limited and further collection of canine stool samples in the present performed, we conclude that there is currently no evidence for autochthonous transmission of CE in this Central African tropical ecosystem.
The taenid tapeworm *Echinococcus multilocularis* is the agent responsible of the alveolar echinococcosis which is the main and most severe parasitic disease in humans in Europe. Due to the assumed wide expansion of the fox densities all around the old continent within the last twenty years this parasite has known a huge spread either in a fox prevalence point of view or in a geographical issue. Some northern countries have yet recently discovered its occurrence on their territories.

If it is well known and described in Western Europe for ages the spread of echinococcosis in foxes sounds to progress toward all directions from its ancient endemic area. Furthermore, cities are now currently colonized by red foxes (*Vulpes vulpes*), which are considered as the main definitive host of this zoonotic cestode. The risk of transmission to humans is nowadays of particular concern where high fox populations overlap with high human populations.

Actually it has been shown that annual mean number of human cases occurring in most of the European countries has been increasing almost in the same time.

Therefore this zoonotic expansion, thanks to the improvement of lab technics, should be surveyed and monitored regularly all over the European statement. For instance a new large scale investigation should start at the beginning of 2016 in France 10 years after the first campaign of monitoring over the same area, using the same methods.

Meanwhile it belongs to national authorities and to scientific teams in every country to try to find the best strategy to control this expanding parasite. Several experiments have been implemented in different countries in order to make the parasite disappear from some territories. Deworming foxes either in rural or urban areas was the main assay. The results are mixed and generally depend on the status of rodent (intermediate hosts) densities as reservoir. Furthermore they sound to be of long term concern to get some efficiency.

Prevent echinococcosis expansion could also pass through the control of fox populations around cities in very high endemic areas. It appears that beyond the ethical issue of such a process the results could be worse and opposite to those expected.

Despite those very interesting experiences carried out to and fro in Europe it seems that the parasite is very difficult to control in fields. If it is not in fields that the control could be planned out, maybe it is worth to improve communication and mass information strategies. That represents a huge task that must be worked out after having assessed what the population needs to know in order to adopt healthy behaviours.

As a conclusion that’s our duty to get epidemiological and technical data within regular time intervals which allow the health authorities to inform accurately populations about the increase or the spatial evolution of this highly anxiogen risk. Than to plan and carry out appropriate mass communication.

**Innovative strategies in the cystic echinococcosis control program in Uruguay**

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Cystic echinococcosis caused by the *Echinococcus granulosus* metacestode is endemic in Uruguay. The Hydatidosis Control Program is carried out by the National Zoonosis Commission which is inter-institutional, facilitating coordination and implementation of strategies. A situation diagnosis performed in 2004 and 2005 indicated that 6.4% of the establishments had dogs infested by *E. granulosus*. Sonographic studies showed human prevalence levels of 1.2% in at-risk areas. Considering these data, in 2006 a new control program was designed. The lines of action developed in areas of high risk health and socio-economic characteristics (scattered rural population, small villages and urban areas considered as of “critical context”) have been: canine echinococcosis diagnosis using CoproELISA, sonography screening in humans, determination of the rate of parasitism in ovine and bovine cattle according to data from slaughter houses with follow-up of geographical origin. Treatment with praziquantel 5 mg/kg per os every 30 days was maintained and wide range anthelmintic drugs were introduced in areas of risk for other geo-helminthiasis (toxocariasis, ancylostomiasis). A program of canine population control has been developed through voluntary cestration of dogs of both sexes. From 2008 to 2013, 117.961 surgical sterilizations of dogs were performed. Regarding human hydatidosis, from 2008 to 2013, 87.536 sonograms were performed. Results from 2008 showed a hydatidosis rate of 6.5/1.000 inhabitants over 23.763 people studied in at-risk areas, while in 2013 2.0/1000 cases were found in 11.473 people examined. Sonogram diagnosis and educational activities were carried out as interdisciplinary and inter-institutional Health Days. In 2008 the number of positive dogs in small villages and suburban areas was 9.9%, descending to 1.6% in 2013, a statistically significant difference (p<0.0001). In scattered rural areas, there were 10.2% positive dogs in 2008, and 1.6% in 2013 (P<0.0001). Cattle parasites according to slaughter houses data throughout the country, decreased from 7.05% to 5.7% in bovines, from 2009 to 2013 and from 5.9% to 3.6% in ovine, during the same period. The Hydatidosis Control Program in Uruguay approaches its task within a Primary Health Care framework. Hydatidosis control requires long-term strategies. Results shown to this date suggest that Uruguay is on the right path for its control, needing continuous assessment and monitoring.
Cystic echinococcosis (CE) is highly endemic in western China. The China CDC reporting system revealed a total of 10,790 CE cases from 2004 to 2008 with more than 95% of the patients from Xinjiang, Gansu, Ningxia, Qinghai and Sichuan with some areas the morbidity rate of the disease reached an average of 4.5-12%. In Xinjiang, the high endemic situation is due to poor economic development, limited community knowledge of CE, a small-scale animal production distributed in the village houses, home killing of livestock and the feeding of dogs with uncooked offal. These features remarkably promote the transmission of E. granulosus and impact on the control of the diseases.

There are two kinds of farming communities in Xinjiang, China. One is based on planting agriculture activity with houses permanently settled (settled villages) and another is nomadic sheep farming communities (nomadic communities). To control CE in the settled villages, a pilot control program based monthly dosing of dogs using baited praziquantel (PZQ) tablets by village hydatid disease control officers (VHDCO) was an efficient and highly cost-effective measure. Given that sheep farmers in nomadic communities are moving to drive their animals from Summer pasture to Winter pasture via Spring/Autumn pasture, the baited PZQ tablets were given by VHDCO for 4 times during vaccination and treatment process in Spring and Autumn pasture and the rest 8 times of dosing dogs were carried out by the dog owners though mobile reminding by the VHDCO. The control measure is simple, affordable and practicable for these rural communities in reducing high endemic CE to less than 1% prevalence in sheep. As a result, the control program of dosing dogs every month has been expended over 150 counties in western China. Control progress was significantly effective in terms of reduction of prevalence in sheep and dogs.

The New Zealand hydatid control research programme started in 1958. Gemmell published in 1966 that protective antigens did occur in the oncosphere or the developing cyst. By 1970, Heath and Smyth had developed the methodology for in vitro culture of oncospheres. In 1975 a dog isolation unit was built in New Zealand that safely supplied E. granulosus eggs for the next 30 years. By 1994 the antigenic polypeptides of E. granulosus had been analysed and tested for protective ability, after a large scale analysis of adjuvants. In Marshall Lightowlers's lab various recombinant iterations were created and then tested in New Zealand. One clone, EG95, showed the best protection and this was sequenced and patented.

The world-first anti-hydatid vaccine was published in Nature in 1996, using QuilA as the adjuvant. Best technology for 10 litre production using GMP was followed by 1000 litre production of the vaccine in New Zealand and registration of the vaccine for commercial use. From 1997 field trials were conducted in Xinjiang, China, using vaccine made in New Zealand. By 2004 the first large-scale production of vaccine occurred in Beijing. Safety testing of the Chinese vaccine was completed by 2006 and then the registration process began, ultimately receiving the registration certificate in 2007. A new factory was built in Chongqing in 2011 and vaccine was ready for commercial use.

Heath provided vaccine for testing in Chile in 1996 and Lightowlers provided laboratory vaccine for a field trial in Argentina in 1999 and again in 2007 and 2009. The vaccine technology was taken up by Tecnovax, Buenos Aires in 2007. The Argentine Government has prepared an action plan for hydatid control, including the vaccine. Peru is keen to use the vaccine for the highland sheep farmers. Large scale field use of the vaccine is planned for the Pehuenche Indian tribes of Chile.

Sixty six other authors have contributed to the project. After 55 years the initial biological trials have been transformed into preliminary commercial use in the field.
Cystic echinococcosis (CE, hydatidosis), caused by the larval stages of *Echinococcus granulosus* sensu lato, is an important zoonotic infection in the world. In Romania, CE continues to pose significant problems of public health and causes important economic losses.

Several options for the control of *E. granulosus* have been thoroughly applied in different parts of the world, with various degrees of success. For Romania, several attempts of control, which come from back in the past (during the period 1959-1965) or more recently (2000-2004), were based on large or limited regional programs, with good results but on short-term. Nonetheless, there is considered that due to a complex of factors (ecological, epidemiological, social and political etc.), the eradication of CE is not achievable for Romania.

Therefore, a horizontal approach for control of this disease, including public health education, the improvement of slaughter hygiene and meat inspection and sanitation measures is currently addressed. Among the main veterinary public health activities, which are included as official actions in the Strategic Program of the National Sanitary Veterinary Authority of Romania, are those relating to the meat inspection of bovines, pigs, sheep, goats, equines and cervids intended for human consumption, carried out in authorized slaughterhouses by veterinarians. However, a big issue for the epidemiology and control of CE in Romania is represented by the animals raised and slaughtered in the traditional small households, where the control is based mainly on the education of people. Subsequently, infected organs are not always properly destroyed. Additionally, specific control measures including registration of all owned dogs, stray dog control, spaying of bitches, have been implementing very recently. The process is undergoing slowly and consequently some measures are facing with public animosities.

However, all the above measures should be complemented by other actions based on legislation, targeting to the interruption of parasite transmission. So, a large involvement including veterinarians, medical doctors, government officials/employees, as well as dog and livestock owners is required for an effective control of CE.

In Europe, parasitic diseases have been, are and will remain emergent. Animal treatment ensures animal and human health and welfare. Echinococcosis/ hydatidosis is a parasitic zoonosis, caused by the genus *Echinococcus*. This parasitosis represents an important public health problem in most parts of the world, in spite of the progress in the domains of surveillance and control. Until now, molecular genetic studies have defined three *E. granulosus* genotypes in Romania: G1 (common sheep strain) in sheep, cattle, pigs, red deer and humans, G2 (Tasmanian sheep strain) in cattle and sheep and G7 (pig strain) in pigs and wild boar.

The therapy of echinococcosis in definitive hosts is essential, being an important stage in the cystic echinococcosis control programme, the main way of preventing the disease. Currently, the anthelmintics of choice in echinococcosis in carnivores are praziquantel, nitroscanate and epsiprantel. Praziquantel, an isoquinoline-pyrazino derivative, acts by changing cell membrane permeability and leads to the decay of parasite skin. Praziquantel is known to be a molecule with high efficacy against cestodes, particularly in infection with *Echinococcus* species. Deworming of carnivores is performed in the prepatent phase, with repetition at 6-8 weeks, in *E. granulosus* infestations and at 4 weeks, in *E. multilocularis* infestations.

Within the cystic echinococcosis control programme in Romania, the effect of two commercially available praziquantel containing drugs (Aniprantel, VimSpectrum, Tg. Mures and Prazicest, A&S International 2000, Bucharest) was assessed in the control of intestinal echinococcosis in dogs. The activity of Aniprantel (5 mg/kg body weight, per os) on *Echinococcus* spp. was tested in 20 dogs compared to an untreated control group. The dogs were tested before and after therapy for *Echinococcus* spp. infection using two methods: coproparasitologic exam and copro-ELISA. The efficacy of the drug for *Echinococcus* spp. turned out to be 100%.

The effect of Prazicest (0.2 ml/2.5 kg body weight, subcutaneous administration) on *Echinococcus* spp. was evaluated in 30 dogs from urban and rural environments compared to untreated controls. The results of the post-therapeutic coproparasitologic examination showed no copro-eliminations of oncospheres, but it showed eliminations of nematod eggs, the efficacy of this product being maximal against cestodes.
Focused assessment with sonography for echinococcosis (FASE) training course: impact in Río Negro Province, Argentina

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Cystic echinococcosis (CE) is endemic in Río Negro Province, Argentina. In 1980 we started a control program. The first ultrasound (US) screening carried out in the world in 1984, in Pilcaniyeu, Río Negro, prevalence rates of 5.6% founded in children from 6 to 14 years old. In 1997 we choose US as the preferred method for abdominal CE screening due to better sensitivity and specificity in comparison with serology. To overcome the shortage of radiologists, in 2000 we developed Focused Assessment with Sonography for Echinococcosis (FASE) training course for general practitioners with no previous experience with ultrasound. The aim was to describe the training system and evaluate the impact of implementation of this strategy for early diagnosis of CE. FASE course lasts 2 days and a total of 20 hours of continuous medical education, with theoretical contain as epidemiologic, clinical, diagnostic and treatment aspects and 2 practical assignments (one group of children apparently healthy and another with known patients with CE). Since 2000, we conducted 15 courses, 13 in Jacobacci and 2 in Menucus, both towns in highly endemic areas of Río Negro. Also we offered the course in provinces of San Juan, Neuquén and Chubut. After the course, trainees were able to carry out autonomous US surveys under the supervision of the referral trainer. We have defined a proposal treatment according to type, localization and size of the cyst of asymptomatic cases: watch and wait, anti-parasitic treatment or surgery. From 1997 to 2014, our trainees carried out 43,177 ultrasound scans with 192 new cases without false positives. US for CE screening can be successfully taught to non-specialists in radiology. This FASE course has allowed the screening in a large population in remote endemic areas with persistent levels of transmission and overcome the barriers presented by the great distances to tertiary referral centers and by the shortage of radiologists in rural areas. The skill of local practitioners to screen for CE using US has enabled the treatment and/or follow-up at local level of those patients who were treated with albendazol or when watch and wait were indicated and refer only surgical cases. It also saved local residents from costly travel time and missing work and avoided unnecessary referral to tertiary centers. US for CE screening proved to be an efficient and low cost intervention tool for both the community and health care system.

Treatment of liver cystic echinococcosis. Nomenclature and therapeutic options

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Human Echinococcosis is a zoonotic infection caused by larval forms (metacestodes) of tapeworms of the genus Echinococcus found in the small intestine of carnivores. Although there are different species described, only five of them – E. granulosus, E. multilocularis, E. oligarthrus, E. vogeli and E. shiquicus – are formally recognized currently as taxonomically relevant. Cystic Echinococcosis (CE) is the disease caused by E. granulosus and it is the most frequent. The presentation form of the larval phase is the development of cysts, called hydatid cysts, in the intermediate hosts. Despite the cysts can be localized in any organ, liver localization is the most frequent (60 - 70%).

This presentation will focus on CE anatomical and surgical nomenclature, once I have frequently noticed, both in published articles and also in communications during scientific meetings, the use of wrong designations concerning the hydatid cyst, which is the larval phase of E. granulosus only. It is not a different nomenclature but the use of inappropriate terms which is based on incorrect concepts, sometimes related to wrong translations from French to English. On the other hand terminology like hydatid disease, hydatidosis or hydatidology is used to designate the Echinococcal diseases in general, which is not correct and is inacceptable. So, these designations shall be avoided and abandoned.

It is pertinent to remember some important points related to the hydatid cysts and, above all, to clarify some aspects concerning its nomenclature in order to understand better its definition and composition, the therapeutic options, particularly the different surgical approaches in the liver locations. For many years, surgery has been the only treatment available for the CE of the liver. Nowadays, we have two more approaches: anti-parasitic drugs (like benzimidazole carbamates) and percutaneous puncture. The option is based on US images, which offer the possibility of knowing the parasite evolution and its activity stage, according ecographic signs, sometimes pathognomonic, and allow choose the ideal treatment.

Keywords: cystic echinococcosis, hydatid cyst, cyst sterilization, hydatidectomy, cystectomy
The place of non-radical surgery for cystic echinococcosis

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Introduction: Although there is a high volume of medical literature regarding liver hydatidosis, the high level evidence coming from Randomized Controlled Trials is lacking.

Objective: The aim of the current study is to describe the peripartum outcomes of cystic echinococcosis and to systematically review the literature about the subject. Method: Retrospective study of patients with hydatid disease admitted between January 2014 - July 2015 in an Emergency Hospital of Bucharest, a tertiary university affiliated center.

Results: Our 18 months data included 33 patients, 56% female, 25 (75.8%) from open group (OG) and 8 (24.2%) from laparoscopic group (LG). We do not find any statistical significant difference between OG and LG regarding age (OG/LG=45.6/30.8, P=0.598), diameter of the cyst (OG/LG=13.6/10.1, P=0.246), in-hospital stay (OG/LG=15.4/9.1, P=0.106), in-hospital costs (OG/LG = 8558/8507, P=0.598), intra-operative visualization of a biliary tract communication (P=0.16), intra-hepatic localization of the cyst (P=0.578), postoperative morbidity according to Clavien-Dindo classification (P=0.729) and postoperative biliary leakage (P=0.701).

Conclusions: A minimally invasive non-radical approach for cystic echinococcosis is safe, with a relative contraindication for minimally invasive approach for central cysts, thick walled cysts and recurrent disease.

Keywords: surgical approach, cystic echinococcosis, systematic review

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Percutaneous treatment of type III (CE 2/3b) liver hydatid cysts: comparison results of a new modified catheterization technique (MoCaT) with PAIR and catheterization

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Purpose: To introduce the results of a new technique called modified catheterization technique (MoCaT) for percutaneous treatment of CE 2 and CE 3b and to retrospectively compare the results of puncture, aspiration, injection and reaspiration (PAIR), catheterization and MoCaT techniques.

Materials and Methods: 73 patients with 75 CE 2 and CE 3b who underwent percutaneous treatment between March 1991 and August 2008 were included. Informed consents of all the patients and approval of the ethical board were obtained. Ages of the patients ranged between 6 and 79 (37 Male, 36 Female). 23 (30%) of the cysts were treated by PAIR, 26 (35%) by catheterization technique and 26 (35%) by MoCaT. The results of three techniques were compared statistically.

Results: Cyst size reduction in all the patients was found to be 61.12% (ranging 5% and 100%). Recurrence occurred in 15 of the patients treated with PAIR (n: 11 [47.8%]), catheterization (n: 3 [11.5%]) and MoCaT (n: 1 [3.8%]) techniques. The recurrence rate was not significantly different between catheterization and MoCaT (p>0.05) whereas with the PAIR technique, significantly more recurrences developed than the other two techniques (p<0.05). 13 (17.81%) major and 15 (20.55%) minor complications were developed. Major complications were observed to be significantly fewer with PAIR technique; difference between catheterization and MoCaT was not significant.

Conclusion: Treatment by MoCaT of CE2 and CE3b seems a safe, reliable and efficient option as it is associated with a lower recurrence rate when compared with the results of other techniques.

New tools for diagnosis with a multidisciplinary approach

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The neotropical echinococcosis represents a severe medical problem in South America. To date, gold standard diagnosis technique is measurement of rostellar hooks size and shape. This is a very reliable test, but is restricted to samples with parasite material observable. Some efforts have been made to overcome this diagnostic difficulty in those situations. We designed two set of primers to specifically identify causative agents of neotropical echinococcosis. Preliminary tests with human and animal samples identify by PCR DNA from E. vogeli and E. oligarthrus positively. Specificity of reaction was also tested and is currently under validation process. These are the firsts PCR reactions that could differentiate samples of E. vogeli and E. oligarthrus, zoonotic species of importance to public health in regions of South America. Validation of this technique will confirm a method with no further sequencing required, making easier the identification in samples with poor conservation process or with no chance of morphometrical examination. The Neotropical Echinococcosis Team of South America and Pan Amazonia is working together to find and test new diagnostic approaches and transfer all those tests to all areas in which are required.
Secondary pleural hydatidosis after liver cyst percutaneous puncture

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Aim: Hydatidosis is a parasitic disease common in Maghreb countries. Usually the lung cysts are single and generally primitives.

The primitive nature of the pleural Hydatid disease is rare and difficult to prove. Secondary pleural hydatidosis is usually produced by intrapleural rupture of a hydatid cyst of the lung, by primitive or iatrogenic way, especially after percutaneous puncture.

So, these patients who present with secondary pleural hydatidosis (SPH) forms with significant morbidity and mortality rates will require a complex management. The purpose of this work was to report these cases and their specific management.

Methods: During the last ten years we have operated 23 patients who had presented SPH after endoscopic treatment. We do not use percutaneous puncture in our department, but we have operated the patients enrolled in this study in other surgical services.

Results: The SPH essentially affected young men. 12 patients had extrapulmonary localization (mainly liver), 5 patients had bilateral lung cyst. All patients were beneficiar of classic surgery, by thoracotomy. In our series the morbidity was 29% and the mortality was 5%.

Conclusion: The SPH is a surgical pathology whose prognosis is bad. The important criterion is a more simple immediate postoperative course. The quality of the initial treatment of the lung cyst is very important to avoid seeing in the future this kind of complications.

Keywords: secondary pleural hydatidosis, percutaneous puncture, surgery

Non-surgical treatment of complicated hydatid cyst of the liver

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Hydatid disease affects most commonly the liver. Up to one-third of the patients with hepatic hydatid cyst present with complications. Intrabiliary rupture is the most frequent complication. The fistula can be occult with no biliary ducts dilation (37%) or can be a frank rupture causing biliary dilatation, which can induce cholangitis or pancreatitis (3-17%). Making the distinction between these two types is important for treatment strategy, which includes surgical, endoscopic and ultrasound guided percutaneous drainage.

The classic treatment for hydatid cyst with rupture into the biliary tract has been surgery; however, persistent postoperative external bile drainage is a serious complication, occurring in 3.8% to 27.5% of cases, that often necessitates reoperation. Other complications include infection within the residual cavity; recurrence or dissemination can also occur. Now, other therapeutical options can be used. Endoscopic sphincterotomy (ES) with or without stenting was reported to have a good efficacy. After sphincterotomy, either a Dormia basket or an extraction balloon catheter can be used to remove the daughter vesicle material from the biliary tract. After internal drainage using sphincterotomy, generally, the patient underwent elective surgery for treatment of hydatid cyst. In some patients ERCP could successfully employed to evacuate biliary daughter cysts and, eventually, to irrigate by nasobiliary drain hypertonic saline into the main cyst resulting in complete cure of the disease. Nasobiliary drain with continuous suction led to healing of a fistula that had not resolved after ES alone. Our strategy to treat is different according to the type of fistula. In case of occult fistula the treatment was based on percutaneous drainage alone, associated in second line to endoscopic biliary sphincterotomy and clearance of biliary duct in case of failure. In case of frank fistula the first line treatment was endoscopic alone, associated in second line to percutaneous drainage in case of failure. Surgical management was used in third line. In all cases Albendazole therapy was used for 3 months. Using these strategies we have treated 47 cases. The treatment was successful in 80.8 % of cases with a Mean time follow-up: 48 months (range: 10-110 months). No major complications were seen related to the procedures. In our experience non-surgical treatment for cyst with biliary fistula was an efficient and safe strategy, challenging the surgical standard therapy.
Treatment of non-complicated lung cysts
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Aim: Hydatid cyst disease is a parasitic infestation that is endemic in many parts of the areas with sheep and cattle farming and it is still a real public health problem in the world. The lung is the second most frequent localization. It’s usually asymptomatic and discovered incidentally.

There are several ways to treat non-complicated lung cysts. Currently the only curative therapeutic option is surgical excision; the medical treatment is reserved to inoperable patients or unresectable cysts. The percutaneous puncture technique is easy, but it poses the risk of draining into the pleural cavity. The video-assisted thoracoscopic surgery has very specific indications. It is mainly used for the treatment of small peripheral cysts especially in children.

Methods: We reviewed the records of 3007 patients operated for pulmonary hydatid cyst in our department during a 32-year period (1983-2014).

Results: 69% of the patients had non-complicated lung cysts. The most frequently technique was conservative surgical treatment used by thoracotomy. In our series the morbidity was 7% and the mortality was 1%.

Conclusion: Hydatid disease is benign when it is very limited, but it should be treated seriously in the initial therapy of the lung, in order to avoid complications and recurrences of the infection.

Keywords: non-complicated cysts, lung, surgery.

An integrating approach to the control of echinococcosis: the South American Initiative
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The South American Initiative (the Initiative) for the Control of Cystic Echinococcosis (CE), built on the successful Southern Cone Subregional Project for the Control and Surveillance of Hydatidosis, was launched in 2013 to include additional country participants and expand its scope to incorporate neotropical echinococcosis (NE). The Initiative gathers officials and academics from Argentina, Brazil, Chile, Peru and Uruguay, under the Secretariat of the Pan American Center for Foot-and-Mouth Disease (PANAPSA), a specialized center of the Pan American Health Organization (PAHO/WHO). Echinococcus granulosus is the parasite responsible for CE/hydatidosis, a zoonosis prevalent in some areas of South America, with an incidence of ~5000 human cases every year and a large social and economic impact on the region’s economy that contributes to the chronic underdevelopment of the populations most affected. In contrast, polycystic NE, caused by Echinococcus vogeli and Echinococcus oligartus, is an emerging zoonosis with over 200 cases reported to date in 14 countries of the region, a high case fatality rate (~29%) and is prevalent primarily in vulnerable populations. The main goal of the Initiative is to prevent the occurrence of CE and NE and improve the health of the populations exposed to the two conditions, by means of enhanced advocacy, communication, education and coordination of surveillance and disease control programs across the region. The Initiative meets once or twice a year and is organized into working groups, each with specific products. In its first two years of operation, the Initiative delivered on a number of fronts: i) a comprehensive online course for officials engaged in CE control programs, ii) facilitated RT-PCR laboratory trainings for researchers from three countries, iii) a guide (in preparation) on surveillance and control to standardize approaches and iv) the first proficiency testing exercise (ongoing) between five national reference laboratories, among others. We will present these products at the conference and more importantly discuss the processes that support the work of the Initiative. We will also share the recently published regional epidemiological report on CE and NE. The report collects and compares national data from the five countries of the Initiative for the first time and it is an important milestone towards improved knowledge of the real burden of CE and NE in the region.
Echinococcosis (Echino) is a neglected zoonotic cosmopolitan parasitic infection, in domestic life were dogs are definitive host, 70-90 percent of stray dogs in Sudan were infected. In livestock 35 percent of camels, 10 percent of cattle and sheep harper the intermediate host of Echino. In wildlife 7-9 percent of foxes and a case of loin Echino have been identified. In hospital based study in Khartoum Pulmonary, Echino is almost half of the cases presented followed by liver. Genotyping of human Echino cyst sample following surgery of the lungs, liver and other sites were is genotype 6 (G6). In a large series of camels exported from Sudan to Egypt, in about half a million of slaughtered camels for meat production, the main organ affected is the lung. This is the same finding at the camel slaughter house in Tambool central of Sudan were lung to liver involvement is 12:1. Echinococcus granulosus G6 has been found in all human lung cases. The definitive pre-operative diagnosis is difficult; ultrasound is not useful, advanced imaging always needed. Percutaneous biopsy is not a routine procedure, mini surgery of the chest is the most appropriate procedure, associated benign pathology was found in many cases. Apart from parenchymal lung Echino, subcutaneous including chest wall and visceral pleura as well as malignancy in some case can be found. Multiple lesions in the lung, uni- or bilateral, are not uncommon. Following surgery, definitive diagnosis is simple, PCR is useful for unusual sites and epidemiology. Outcome of surgery is excellent which is followed by three month course of chemotherapy (Albendazole), follow-up of cases for many years without recurrences and complications.

The success of any treatment is strictly related to the right diagnosis of the disease and its evolution stage. Lung CE is not excluded from this principle, mostly because it can mimic many diseases, according to its evolution: complicated or not. The differential diagnosis of non-complicated lung CE should be done with many conditions, like: lung TB (tuberculoma, infiltrate, cavern filled with material), benign lung tumors (hamartoma, lipoma, fibroma, angioma, leiomyoma etc.) malignant bronchopulmonary tumors (primary or metastasis), tumors and cysts of mediastinum (dermoid cyst, gastroenteric cyst, neuroenteric cyst, mediastinum hydatid cyst, lipoma, neurinoma, fibroma), congenital lung cysts, bronchogenic cyst, pleuropericardic cyst, infected aerial cyst), other lung malformations (lung sequestration), aortic aneurism, pleural diseases (axillary or interlobar encysted pleurisies), tumor, inflammatory or cystic parietal diseases (sarcomas, cold abscesses, fibrocystic dysplasia), diaphragm and liver CE.

Complicated CE can mimic other conditions, according to the type of the rupture: bronchial rupture – evacuated lung abscess, infected aerial cyst, emphysema, cavernous TB with level, diaphragm hernia (gastrothorax); in pseudo tumor form (with incarcerated membrane) the differential diagnosis should include all solid thoracic neoformation; rupture in pleura – spontaneous pneumothorax, para- or meta- pneumatic pleurisies, neoplastic pleurisies, mesothelioma.

In spite of paraclinical methods, laboratory tests or imaging investigations (CT, IMR, ultrasonography), some types of CE, mostly complicated, can be assigned as another lung condition. In our statistics, even histological diagnosis through bronchial biopsy revealed erroneously the presence of a squamous carcinoma, knowing the fact that many times inflammatory lesions, consequent to the germinial membrane, can mimic the lung cancer.

The aim of this work is to underline the necessity to precise not only the diagnosis of the disease, but also the one with surgical recommendation. In this way, the number of the patients treated by chemotherapy for incarcerated cystic membranes and those with long term TB treatment (liver and kidney toxicity!) for residual cavities after evacuated CE will decrease. It is also perfectly valid that reciprocally, we have lung cancer treated with antiparasitic treatment.
Despite excellent public health systems, alveolar echinococcosis (AE) has been constantly expanding in Europe within the past two decades, including a significant expansion into Northern, Eastern and Western Europe. Emergence of E. multilocularis endemicity in fox populations has been confirmed in many European countries, including the Baltic States, Poland, Slovakia, Romania and Slovenia. Furthermore, countries previously documented to be free of E. multilocularis (e.g. the United Kingdom, Ireland, Malta, Norway, Finland) are taking great efforts to assess the risk of introducing E. multilocularis into their countries through imported dogs and wildlife animals. Increased traffic of pet dogs and relocation of wildlife have already sporadically contributed to the spread of this zoonosis. An increase in the incidence of human AE cases has already been documented in some areas. In Switzerland, for example, an average twofold increase of the annual incidence was reported between the periods 1993-2000 and 2001-2005 (Schweiger et al., 2007). Observations in France and Austria revealed similar trends. In some of the countries of Eastern-Central Europe, which were not considered to be endemic areas, a limited but steady increase in number of human cases has been observed and in the Baltic countries, such as Lithuania, a tenfold increase of the AE-incidence was reported in 2009(12). Such trends all over Europe may not only be due to the increase in infection potential but also other causes, e.g. to the growing number of patients under immunosuppressive therapy with a significantly higher risk of developing AE (Chauchet et al., 2015). High environmental contamination pressure for intermediate hosts is also illustrated by the occurrence of AE in accidental intermediate hosts that usually do not take part in the parasite cycle, such as dogs, pigs and primates.

The very nature of the life cycle of AE, with predominantly wildlife as intermediate (rodents) and definitive (fox) hosts, precludes eradication. So far, long-term baiting of foxes with appropriate medication is the most effective tool to locally decrease the environmental contamination and prevalence in wild hosts (and consequently in dogs as well) in a significant, although temporary, way. Furthermore, dogs with particular infection risk (free roaming and with access to rodents) can reach remarkably high prevalence of infection with E. multilocularis. A monthly deworming scheme for domestic dogs with access to rodents represents an effective measure to reduce the risk of infection in humans, provided that an appropriate drug such as praziquantel is used. The European Scientific Counsel on Companion Animal Parasites (ESCCAP) has started informational campaigns in Europe in that respect. Currently, treatment options for AE are few. Surgery is reserved for early stage treatment when lesions can be completely resected with a safe margin of unaffected tissue and no distant metastases. Drug treatment for all other cases has its own limitations. Only two closely related drugs (albendazole and mebendazole) can be used to treat AE and both significantly contributed to a relevantly longer survival of AE patients as compared to the situation prior to drug treatment. Nevertheless, they rarely eliminate E. multilocularis and thus life-long treatment is required to inhibit or at least suppress parasite growth in AE patients who could not benefit from radical surgery. Based on the long-term clinical experience of Swiss, French and German clinical AE centers, standardized AE diagnosis and treatment protocols can be developed, validated and disseminated. Networking with reference centers in AE-endemic countries including Baltic and Central-Eastern European countries is essential to meet the threat of increasing numbers of AE patients and to achieve commonly agreed standards. Specific immunological tests in combination with high-performance imaging techniques promise substantial improvements in early diagnosis, essential for curative treatment, as well as in staging and follow-up of patients. Furthermore, standardized registration and follow-up protocols are essential to properly assess the epidemiology of the disease and its trends in all European countries and to prepare the ground for multi-centric clinical trials to formally test new treatment options. An extended patient registry at the full European level would not only significantly contribute to improve the clinical management of AE, but also to better delineating areas and populations at risk to test new prevention strategies and in promoting disease awareness in these populations.

The currently observed trends of E. multilocularis infection in the European fox and dog populations and the expected increase of annual case numbers of human AE in many areas of Europe strongly advocate for scaling up research that can improve the fields necessary to yield better management of this infectious health problem in Europe.
Neglected tropical diseases (NTDs) are still widespread and create a substantial burden in China. About 90% of all cases of echinococcosis in China are cystic echinococcosis caused by *Echinococcus granulosus*, whereas the remainder is from alveolar echinococcosis, which is caused by *Echinococcus multilocularis* and produces more severe pathological changes. The direct economic burden for human CE was 11646.48 RMB per person and the indirect economic lost was 11200.65 RMB per person. China harbors more than 90% of the world’s burden of alveolar echinococcosis.

To effectively reduce the burden of echinococcosis, an integrated control approach has been launched, consisting of treatment for patients and dogs, information, education and communication strategies (IEC) and improved slaughter management. The goal for 2015 is to reduce the infection rate in children by 60% compared with 2005. CE has the highest prevalence (averaging up to 5% in endemic areas of the Tibetan plateau and in Xinjiang and Ningxia Autonomous Regions) and in the 1990s, national and international cooperative research work confirmed the extent of the disease, geographically and within specific populations, including minority ethnic groups (Tibetan, Mongol, Kazakh, Uyghur and Hui). This issue has received much attention from Chinese public health authorities, including a special national program coordinated locally by the regional/provincial Centers for Disease Control, which includes specific coverage by the national public health insurance for human cases, as well as veterinary public health measure. During 2005-2006, human echinococcosis prevention, control and treatment network has been initially setup in Xinjiang. The network Included 29 hospitals in 2008 and provided surgical training, scientific research, and surveillance for human echinococcosis. Appointed hospitals of surgical treatment for human echinococcosis covered more than 70 hospitals in 7 Provinces/Regions in China. The first affiliated hospitals of Xinjiang Medical University (FAHXMU) provided standardized training, supervising and consultation for hydatid prevention, control and treatment. Tele-Medicine center was established in FAHXMU in 16th April, 2008 and played crucial role in training, prevention, control and treatment. Currently, Tele-Medicine network has covered more than 90% counties with 158 more hospitals and provided consultation for more than 3000 hydatid cases among 70,000 hydatid cases. Tele-Medicine consultation and remote live demonstration come into practice with Inner Mongolia, Gansu province and Aba County, Sichuan province and yielded initial success. The setup of network and application of Tele-Medicine system made further improvement in prevention, control and treatment of hydatid disease in appointed hospitals and enhanced the information share among different hospitals and to large extent lessen the economic burden.

In conclusion, as Chinese National Programme goals set the Reduction of the infection rate in children by 60% by 2015 (Action Plan to Prevent and Cure Echinococcosis 2010–15). Control and treatment measures include: control infection sources and intermediate hosts, information, education and communication strategies; (IEC), patients’ management, improved sanitation and water supply and surveillance with improved and standardized clinical management.
Control of Echinococcus multilocularis in definitive hosts

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In contrast to most agents of cystic echinococcosis (CE), Echinococcus multilocularis is basically a wildlife parasite, whose control requires radically different strategies than control of CE. In principle, the parasite could be targeted by (1) host control and (2) anthelmintic treatment of hosts. Reduction of wild canid populations (by hunting or trapping) to an effectively low level is considered unfeasible or unethical and is environmentally destructive in case of large-scale poisoning of rodent intermediate hosts. Anthelmintic treatment of wild canids can only be done by regularly repeated distribution of baits. Beginning in the 1990s, several small- and large-scale longitudinal studies have been done in Europe and Japan using baits of different types, containing 50 mg praziquantel. The baits were distributed on the ground or by aircraft at various densities (mainly 15-50 baits / km²) and at different frequencies (once per month to once per six months). Despite different environmental conditions (urban vs. rural), the majority of studies demonstrated a drastic reduction of E. multilocularis prevalence in red foxes (which are the main definitive hosts in the study regions). Although parameters varied (level of prevalence decrease, long-term effects), efficacy was apparently linked to short intervals between baiting, high density of baits and the inclusion of settled areas in addition to surrounding rural landscapes. In view of the available data, there appears to be sufficient expertise for practical application of fox baiting as a means of E. multilocularis control. Constraints are high costs and unresolved cost-benefit issues, inconsistent commitment of bait producers and authorities at various administrative levels and legal restrictions (distribution of medicinal compounds in the environment).

Towards a European database on human cases of alveolar echinococcosis

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The French registry of Alveolar Echinococcosis (AE) has included 607 cases registered between 1982 and 2014. Recording is based on voluntary declaration by medical staff in charge of patients (clinicians, surgeons or biologist) through the FrancEchino network. The following pieces of information have been stored: patient’s data (ID, gender, etc), diagnosis, treatments, liver status, staging according to WHO-IWGE classification, risk factors and aftercare. The 607 AE cases currently reported by the FrancEchino network over the period 1980-2014 were hitherto available as Excel® spreadsheets. We are now moving to an interoperable and secured MySQL database with a Web application in PHP format for data visualisation (data mapping) and queries. This data management system is coupled with the CleanWEB platform that allows practitioners such as clinicians the online input of patient data. This database ensures quality control of recorded data, confidentiality and the possibility of connection with other databases. This system will serve as a basis for the construction of a European database on human AE (EurEchino database). To this end, we organised a European workshop in Besançon on the 9th and 10th of April, 2015, in order to share experience about AE data collection and management. Thirty-five participants from Germany, Austria, Belgium, Italy, Latvia, Poland, Romania, Slovakia and Switzerland, with an observer from China, discussed on the best strategy to harmonize the census methods of human cases and adopt the database system set up at Besançon WHO Collaborating Centre. Working groups have been organised around the following topics: (1) Database item definitions: clinical items (diagnosis and follow-up), (2) Database item definitions: clinical items (treatment); (3) Database item definitions: epidemiological items; (4) Case collection methodology and ethical issues; Participants’ / users’ code of conduct. Following this workshop, a minimal list of items to be included in a European-scale database, as well as a case collection framework and ethical rules for common data usage have been agreed upon by all the participants. Recording using this system will start in October 2015, after a last discussion to adjust the item definitions at XXVIth World Congress on Echinococcosis that will be held in Bucharest, Romania (01-03 October 2015).
Immune suppression and AE in experimental animals and in humans

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The influence of immunosuppression on *E. multilocularis* development was shown in mice as early as 1976. Since then, our knowledge of the host-parasite immune interactions has greatly benefited from the use of experimental models of immunosuppression and from the observation of alveolar echinococcosis (AE) in humans with immune deficiency.

Global suppression of cellular immunity induced by immunosuppressive drugs in mice is associated with faster growth of the parasite; similarly, genetically immunosuppressed mice, such as nude mice or SCID mice, are especially susceptible to its development. Conversely, suppression of humoral immunity is not associated with changes in susceptibility/resistance. Suppression of specific actors of the immune response as produced in mice by gene deletion ['knock-out'(KO) mice] have allowed researchers to dissect the mechanisms of host’s resistance/susceptibility. For instance, KO mice for TNF allows faster and better development of *E. multilocularis* on the opposite KO mice for FGL2, a protein associated with the functions of T regulatory cells, are partially protected against *E. multilocularis* growth.

In humans, recurrence of disease and faster growth and dissemination of the metacestode were first observed in the 1980s when liver transplantation became a modality of treatment in severe AE cases. This was also observed in patients with AIDS related to HIV infection. More recently, with the availability of more potent and/or targeted drugs/biological agents, including anti-TNF, there has been an increased use of therapies with high immunosuppressive impacts in diseases such as cancers, haematological malignancies, and chronic inflammatory disorders, and/or after allo-transplantation. This increase is associated with an increased incidence of AE in such patients, with unusual ‘acute’ presentation and fast progression. More frequent negative serology and misleading images at CT, MRI or PET make diagnosis of AE more difficult, especially in the non-endemic areas, which may be source of erroneous treatments. However, the development of ‘pre-clinical animal models’ may take benefit from such observations and use immunosuppressive agents in order to fasten parasite development of AE in otherwise ‘resistant animals’ closer to the human situation than mice. This was recently performed in pigs using the concomitant administration of dexamethasone or of cyclosporine and metacestode injection in the liver, with very encouraging results.

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Advances in immunological diagnosis and follow-up of AE

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Serological techniques help in diagnosing alveolar echinococcosis which is usually suspected by medical imaging. The enzyme-linked immunosorbent assay (ELISA) using recombinant Em18 (rEm18-ELISA) or rEm18 plus the native Em2 antigen purified from *E. multilocularis* larvae (Em2 ELISA (Bordier Affinity, Crissier, Switzerland), are currently being used in most laboratories as a 1st line step. Indirect hemagglutination (Hydatidose Fumouze kit, Fumouze Diagnostics, Levallois-Perret, France) is one of the low-cost screening techniques and the Western blot technique (LDBio Diagnostics, Lyon, France), using a whole *E. multilocularis* larval antigen, is the confirmation technique (2nd line) and may help for species diagnosis. Inter-laboratory standardization of results, however, was not performed until recently.

Accreditation according to ISO 15189 or to other international standards (i.e. ISO/IEC 17025 and ISO 9001) is an essential step for medical labs to provide accurate data for patient diagnosis and follow-up. It addresses the qualifications and on-going skills of personnel, pre-analytical and analytical factors, quality assurance considerations and post-analytical factors. It includes the participation in external quality assessment (EQA) or Inter Laboratory Comparison (ILC) program for each parameter. For the first EQA program (2013) implemented by the French National Reference Centre for Alveolar Echinococcosis (NRC), ten French laboratories were directly solicited. For the renewal of this EQA program in 2014, 21 French laboratories and 5 European laboratories were included. This EQA program led to an increased confidence level of analytical results and to the implementation of a European network of labs involved in echinococcosis diagnosis. Serological techniques also help in assessing parasitic viability. *Em2* + serology and rEm18-based serology, along with Fluorodeoxyglucose positron-emission tomography, are used for treatment monitoring. However, these tools are not optimal and the development of new biological markers able to predict *in vivo* parasite viability is of utmost importance to improve the management of AE. In a recent study, we analyzed the vesicular fluid (VF) from in vitro culture of *E. multilocularis* using an immunoproteomic approach with serum samples from patients “responders” and “non-responders” to ABZ treatment. This comparative approach allowed us to identify 5 VF proteins that appear particularly interesting for the development of an ELISA assay helpful in decision making for discontinuation of ABZ treatment.

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"Echinococcus multilocularis infection in animals" (EMIA): project funded by EFSA to support the review of the Regulation (EU) No 1152/2011

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Introduction: Till now, four EU member states have submitted to EC the documentation supporting the evidence of the absence of Echinococcus multilocularis (EM). Regulation (EU) No 1152/2011 provides that a pre-moveement antiparasite treatment has to be applied to dogs entering these four countries and that a pathogen-specific surveillance programme has to be operated. The Commission has to review this Regulation no later than December 2016 in the light of scientific developments regarding EM infection in animals. The European Food Safety Authority (EFSA) will be requested to provide a scientific opinion on EM infections in animals by November 2015. To assist in this review, EFSA funded the project “Echinococcus multilocularis infection in animals” (2012-2015).

Methods and Materials: Information and data were gathered by means of 8 systematic reviews (SRs) of literature and data. Bibliographic searches, within the 8 a priori protocols were carried out using the following databases: MEDLINE (Medical Literature Analysis and Retrieval System Online), EMBASE (Excerpta Medica Database), SCI SEARCH (Science Citation Index), BIOSIS (Biological Abstracts), CABI (Centre for Agricultural Bioscience International), GOOGLE SCHOLAR. The platform used for this SRs is STN International – Fiz Karlsruhe. Searches were carried out on 5/11/2013 and 11/02/2015. The results of the two searches were combined. The search was restricted to eight languages from electronic databases (English, Italian, Polish, Dutch, German, Spanish and Finnish).

Results: Bibliographic searches identified 10,737 scientific papers of which 5,316 were deleted because duplicates. At the end of the search, 5,421 papers were identified within the eight protocols of which 4,569 were excluded through title and abstract only. Full text papers assessed for eligibility were 813, data were extracted from 443 studies and it was possible to perform meta-analyses on 362 studies. Studies resulting relevant for more than one question were independently analyzed in each Systematic review.

Conclusion: In order to be able to provide a comprehensive and quantitative assessment of EM infections in animals, the current knowledge and data on the epidemiology and risk factors related to this disease were collected in the EU and adjacent countries.

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Distribution and prevalence of Echinococcus multilocularis in animals and importance of the different hosts in the EU: a systematic review

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Aims: This study aimed to provide a systematic review on the geographic distribution of E. multilocularis (EM) in definitive (DH) and intermediate hosts (IH) and the relative importance of the different hosts in the life-cycle (LC) of EM in EU and adjacent countries.

Material and methods: Six databases were searched for primary research publications since 1900 to 2015. The quality of studies was assessed with the Newcastle-Ottawa Scale. Statistical analysis was performed with Review Manager 5.2.

Results: From 2,805 publications, 238 publications were used for meta-analysis. For DH, Finland, Ireland, UK and Norway reported the absence of EM in red foxes (RF). In Norway (Svalbard), arctic foxes showed the presence of EM. 20 countries reported EM in RF, with the following pooled prevalence (PP): low (<1%); Denmark, Slovenia and Sweden; medium (1% to 10%); Austria, Belgium, Hungary, Italy, Netherlands, Romania and Ukraine; high (>10%); Czech Republic, Estonia, France, Germany, Latvia, Lithuania, Poland, Slovakia, Lichtenstein and Switzerland). Raccoon...
dogs (PP 2.2%), golden jackals (PP 4.7%) and wolves (PP 1.4%) showed a higher PP than domestic dogs (PP 0.3%) and cats (PP 0.5%). The high PP in raccoon dogs and golden jackals correlates with the high PP in RF. For IH, muskrats (PP 4.2%) and arvicolids (PP 6.0%) showed similar PP as sylvatic DH, excluding RF. Nutrias (PP 1.0%) and murids (PP 1.1%) could play a role in the LC of EM in areas with medium to high PP in RF. Insectivores and mustelids seem to play no role in the LC of the parasite. EM positive swine were found in Germany and Lithuania. In Svalbard (Norway), Microtus spp. showed = 50% PP.

**Conclusion:** In areas with low PP in RF, no other DH is infected with EM. When RF PP >3%, raccoon dogs and golden jackals could play a similar role as RF. For high fox PP, the wolf shows up as a potentially important DH. Dogs and cats could be irrelevant in the LC of the parasite in Europe, although dogs could be important for parasite introduction into non-endemic areas. Muskrat and arvicolid are important IH and, under specific conditions, also Microtus spp. Swine, mustelids, insectivores, murids and nutrias seem to play a minor or no role in the LC of the parasite. There are few studies on murid rodents; therefore understanding about their role in the EM LC is inadequate.

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### Risk factors associated with human alveolar echinococcosis: a systematic review

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**Aims:** Alveolar echinococcosis (AE) is caused by infection with Echinococcus multilocularis. Humans may be aberrant intermediate hosts and develop the clinical condition of AE, which can be lethal if left untreated. This study aimed to provide a systematic review of the currently available data on potential and known risk factors associated with human AE.

**Material and methods:** Six databases were searched for primary research studies (cross-sectional, case-control, cohort studies and studies with experimental infection) published since 1900 through to February 2015 in eight different languages. The quality of studies was assessed using the Newcastle-Ottawa Scale. Statistical analysis was performed using the software Review Manager 5.2.

**Results:** From a total of 958 publications identified, 379 were excluded in the first step. Of the remaining publications, 73 were assessed as eligible after studying the full text. Data could be extracted from 29 papers and a meta-analysis performed on 17 publications. The following risk factors of potential global relevance were identified: dog ownership, playing with dogs, female gender, age > 20 years and occupation (herding). Other identified potential risk factors were cat ownership, having a kitchen garden, occupation (farming), haymaking in meadows not adjacent to water, going to forests for vocational reasons, chewing grass and handling foxes, whereas particular human leucocyte antigen (HLA) types turned out to be protective.

**Conclusion:** The studies were rather heterogenic with regard to the geographic location, study design and quality of results, not least because of the heterogeneous situation of the disease itself and due to the long incubation period, which makes the identification of risk factors difficult. The results will contribute to draft the review of the current Regulation (EU) No 1152/2011, especially in terms of targeting prevention and control measures against human AE.

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### Allocation of cystic echinococcosis patients to treatment

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There are four management options for cystic echinococcosis (CE): surgery, percutaneous treatment, medical treatment with benzimidazoles and watch and wait or expectant management.

Although they have developed over decades without adequate comparative evaluation of efficacy, effectiveness, rate of adverse events, relapse rates and cost, the use of a stage-specific approach following the WHO-IWGE classification for CE greatly facilitates a more rational treatment (as opposed to “one size fits all”), which in turn reduces risks for the patient and expenditures for the health system.

This presentation will illustrate the stage-specific criteria for treatment allocation in patients with CE of the liver.
Laboratory techniques for the detection of Echinococcus multilocularis in animals and effectiveness of available de-worming drugs: a systematic review

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*SBEAKERS

Aims: The aim of the first systematic review (SR1) was to compare the laboratory techniques available for the detection of Echinococcus multilocularis (EM) in animals to assist in the development of (standardized) test protocols. In the second SR we aimed to give an overview on the de-worming drugs available for EM treatment and to establish treatment protocols (SR2).

Material and Methods: Six databases were searched for publications since 1900 to February 2015. The quality of studies was assessed with the QUADAS-2 (SR1) and the Cochrane tools and the JADAD score (SR2). Statistical analysis was performed with the Review Manager 5.2.

Results: From 4,055 (SR1) and 382 (SR2) publications, only respectively 15 and 12 could be used for meta-analysis. For EM detection in dead animals, the sensitivity of the sedimentation and counting technique (SCT), still regarded the gold standard, is high but depends on the worm burden. Sensitivity of the shaking in a vessel technique is high but this was only based on one paper. Segmented SCT and the intestinal scraping technique (IST) showed, based on respectively one and two papers, lower sensitivity (98.3%, 25-78%) than SCT. The four techniques are highly specific. For EM detection in live animals, sensitivity of faecal flotations and microscopy was influenced by intermittent shedding and specificity was low and does not allow estimation of worm burden. The sensitivity of the coproantigen ELISA is influenced by the worm burden, with an overall sensitivity of 82% compared to the SCT. It showed to be useful in known-infected areas, but less useful in regions with a sporadic or unknown endemicity. Data of copro-PCR assays were especially heterogenic. In many studies, copro-PCR assays were more sensitive than IST or SCT.

Conclusions: From SR1, there is not enough evidence for recommending a diagnostic technique. PZQ is the drug of choice for the treatment of dogs and cats. No recommendation can be given on how long after treatment faeces need to be discarded.

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On track for 2020? Towards the WHO roadmap’s targets for neglected tropical diseases - Echinococcosis control

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Echinococcosis is one of the zoonotic diseases included in the portfolio of the Department of Control of Neglected Tropical Diseases (NTD) at the World Health Organization (WHO). The disease affects neglected, poverty-stricken communities, yet remains a low priority for governments and receives inadequate attention from the international public health community. Where health systems are weak and health coverage is lacking, patients with echinococcosis often do not receive the care they need. Systems for control of the disease through food safety and livestock-keeping are too weak to break the cycle of transmission. Political will is required to address the lack of coordination among the human, veterinary and environmental health sectors and to set up effective national strategies to combat the burden of echinococcosis. High-quality surveillance and data are urgently needed to show the impact and burden of the disease and to support priority-setting and allocation of funds. Much work in endemic countries is needed on integrated control interventions in animals, related to both dog and sheep populations, as well as food safety. Improvement of case detection and case management is crucial.

The WHO Informal Working Group on Echinococcosis has been a union of researchers since 1985. Its initial mandate was to facilitate exchange between interested scientists working in different areas of echinococcosis control. The WHO roadmap on Neglected Tropical Diseases published in 2012 sets a target for echinococcosis of building a validated strategy to combat echinococcosis/hydatidosis and scaling up control and elimination interventions in selected countries by 2020. WHO thanks the valued chairmanship of Professor Peter Kern from 2010 to 2014, and opens the call to identify a new chair and co-chair to carry on the work of coordinating the exchange of knowledge and research to support countries in their effort to combat echinococcosis and building momentum towards attainment of the targets.

WHO collaborates with the Food and Agricultural Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) to foster multisectoral collaboration and strong partnerships with public and private stakeholders. It is only through collaboration with, and determination of, all stakeholders that the control of echinococcosis/hydatidosis can advance.
Monitoring and surveillance programmes for Echinococcus multilocularis in definitive and intermediate hosts in Europe: a systematic review

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Aims: The life cycle of Echinococcus multilocularis (EM) is mainly sylvatic and is based on the predator/prey relationship between definitive (DH, foxes) and intermediate hosts (IH, small rodents). Domestic dogs or cats can also be sporadically infected in Europe. Several epidemiological studies have been published in Europe giving information about the prevalence in wildlife, principally in foxes, however only few data are available on IH. The objectives of this systematic review were to identify and describe the monitoring and surveillance programmes on EM in the IH and DH, to determine their scale in the European Union and adjacent countries.

Material and methods: Six databases were systematically searched for primary research studies published since 1900 to February 2015. The quality of studies was assessed with the Newcastle-Ottawa Scale. Statistical analysis was performed using the Review Manager 5.2. Meta-analyses were performed focusing on the DH (divided in red fox, dog, cat and raccoon dog) and IH (grouped in microrodents and aquatic rodents).

Results and conclusions: The systematic search identified 1316 scientific papers. Through title and abstract analysis, 643 were excluded. After full text papers assessed, only 83 studies met the eligibility criteria in a priori protocol and were suitable for data extraction. It has been possible to perform meta-analyses on 72 studies.

In Europe, fox is the main targeted DH for monitoring /surveillance programmes. Dogs in Europe seem not to be an interesting target species for monitoring /surveillance programmes in countries or areas where the expected prevalence in foxes is ≤1%. Monitoring EM in small rodents is possible, but the meta-analysis results indicated that they aren’t good indicators for EM infection at large scale area. For aquatic rodents, surveillance programmes could reflect the presence of EM in new endemic areas where the prevalence of foxes is around 5%. Concerning the sampling effort, this systematic review highlighted that in low prevalence, foxes are the main and almost the only species targeted for surveillance/monitoring programmes.

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Patients with NTDs: the long way from recognition to evidence-based management

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Neglected Tropical Diseases (NTDs) are mostly diseases of the neglected poor in some parts of the world. Some are “slow” diseases, advancing silently until chronic and difficult to treat disease stages are reached. Cystic and alveolar echinococcosis, neurocysticercosis and schistosomiasis are examples. Scientific interest and investment into NTDs have been disproportionately poor compared to their negative health impacts. This hopefully will change with the London Declaration on Neglected Tropical Diseases (http://unitingtocombatntds.org/resource/london-declaration) and the G7 Leaders’ commitment at their summit in June 2015 to fight against NTDs. A lot remains to be done in view of the limited tools available to diagnose and treat NTDs. Early diagnosis is key but problematic when positive screening is not followed by efficient and safe treatments. On the other end of the range, in advanced disease stages, treatment success is often no longer determined by specific therapy but availability and quality of health services in the area of surgery, anaesthesia and so on. Clinically, patients with NTDs such as cystic echinococcosis remain trapped between the two extremes if no new diagnostic tools and therapeutic strategies are developed. This dilemma is aggravated by the fact that all NTDs are in principle preventable. Research groups working on NTDs are small, even on a global scale. Academically, they are not among the most rewarding research fields. The implications of this on the generation and quality of clinical data, data analysis and diagnostic and therapeutic guidelines should not be underestimated. Motivating and supporting young researchers to take up the challenge of NTD research and to bring in their knowledge of much needed stringent methodology and new research tools will determine what patients with NTDs can expect in the future. The recognition of NTDs by the G7 states hopefully provides financial means and facilitates the implementation of high quality research results.
Treatment of neotropical polycystic echinococcosis

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One difficulty encountered in the treatment of this echinococcosis is that there are no substantial data in the literature to define the best therapy. There are no double-blinded controlled studies that could guide the best approach, because the reported cases do not reach two hundred and most publications are case reports and, in this situation, there is no way to define protocols.

Therefore, treatment is based on the treatment used for *E. granulosus*. However, as often times patients are diagnosed at advanced stages of the disease, in which surgical treatment (gold standard treatment for cystic echinococcosis) is withheld, it is opted for chemotherapy with benzimidazole wherein the albendazole is the most used drug at a dose of 10 to 12 mg/kg/day. That said, the question remains, who to indicate chemotherapy or surgical treatment for polycystic echinococcosis?

Most of the cases we deal in the state of Acre - Brazil - have only liver involvement. The progress in the techniques of liver surgery is increasingly providing more selective and safer surgical procedures, especially in benign diseases, which do not require the resection of a safety margin. Thus, the greatest challenge is the diagnosis at an early stage of the disease, which allows surgery. Moreover, in advanced cases, such as in portal hypertension and in signs of severe hepatic impairment, liver transplantation may be indicated, although it is still an incipient experience.

There are cases with onset only in the mesentery or in the peritoneum. In these, the apparently less complex resection can be problematic in situations that have hundreds of cysts comprising important structures such as vascular pedicles.

The study of each particular case, the risk-benefit analysis, should guide the indication of each treatment modality.

Hydatid disease of the liver: 10 years’ experience with 853 patients

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Selection of the most appropriate treatment to obtain the best results with the lowest rate of recurrence and minimal morbidity and mortality is mandatory for the management of hepatic hydatid disease. We aimed to evaluate the results of current therapeutic methods in the context of a 10-year single-institution experience.

Between 2005 and 2015, 853 patients were treated in “Dan Setlacec” Center of General Surgery and Liver Transplantation by open or laparoscopic surgery. The series included 512 females and 341 males ranging in age from 2 years to 88 years. In more than half of the patients (502, 58.9%) the disease was localized in the right lobe of the liver, in 244 (28.6%) patients was localized in the left lobe, and 107 (12.5%) had cysts in both lobes of the liver. We retrospectively analyzed the preferred treatment modalities, perioperative complications, interventions, recurrences and length of hospital stay. We preferred the open approach in 789 (92.5%) cases and laparoscopic approach in 64 (7.5%) cases. The most common intraoperative complication was intrabiliary rupture of the cyst in 226 (26.5%) cases. Biliary leakage was the most common postoperative morbidity and was encountered in 274 cases. There were four postoperative deaths, due to multisystem organ failure, pulmonary embolism, hydatic membrane pulmonary embolism and respectively vascular fistula with anaphylactic shock and disseminated intravascular coagulation.

In conclusion, selection of the treatment option that provides the best results with the lowest recurrence rates and minimal morbidity and mortality is the key for success in the management of hepatic hydatid disease. The condition of the patient, characteristics of the cyst and the presence of cystobiliary communications are the factors we believe directly affect the outcome.

Status of neotropical echinococcosis

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The neotropical echinococcosis, polycystic, caused by *Echinococcus vogeli*, and the unicystic, caused by *Echinococcus oligarthrus*, are emerging zoonosis in human populations in South America. These two species are different from the others of the genus *Echinococcus* in their epidemiology, life cycle, cyst and rostellar hooks morphology, and for having different approaches to prevention, diagnosis and control.

At this time, 14 tropical South American countries are known to have human and animal *E. vogeli* infections and about 250 human are infected with the parasite. These figures probably represent a small portion of undiagnosed infections, which may give origin to fault perception of mortality and morbidity in health systems. In addition, human migration, international trips with long stays in the visiting countries, as well as ecotourism may alert to responsible organizations, affine and transversal to the country health, to take note of the real distribution of these parasitic infections.
The zoonotic transmission of *Echinococcus multilocularis* in the light of human-wildlife interactions

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The life cycle of *Echinococcus multilocularis* depends to a large extent on the distribution and population dynamics of its wild host species and the predator-prey interplay between the final and intermediate hosts. These factors are in many ways affected by human activities and human attitudes towards wildlife. Correspondingly multi-faceted human-wildlife interactions shape the transmission dynamic of this parasite. E.g. rodent communities can be strongly affected by changes in the agricultural practice and by the kind and intensity of pest control measurements. Fox densities increased strongly due to oral-vaccination campaigns against rabies in many countries during the last decades. In addition foxes are actually not exposed to top predators like wolves or coyotes over large parts of their distribution area. A changing human attitude towards wildlife promotes many people to approach and feed wild animals. Such interactions in combination with the absence of top predators and a lower hunting pressure can modify the “landscape of fear” for foxes. As a result foxes can lose their shyness and therewith explore new food resources in the middle of densely populated areas. This so-called “urban tameness” can facilitate the establishment of the parasites life cycle in the middle of urbanised areas. On the other hand rich anthropogenic food resources areas can lower the predation of foxes on rodent intermediate host and negatively affect the transmission intensity. Such complex human-wildlife interactions should carefully be analysed when it comes to the development of strategies for the prevention and control of this zoonotic disease.

*Echinococcus multilocularis* in different rodent species: heterogeneous transmission dynamics at a small spatial scale

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Different rodent species, mainly of the family Cricetidae, are known to act as intermediate host for *Echinococcus multilocularis*. So far little is known about their relative importance for the maintenance of the parasite life cycle. In the framework of the European research project ”*Echinococcus multilocularis* in rodents” (EMIRO), we compared prevalence rates and parasite fertility in different vole species, namely *Arvicola scherman*, *Microtus arvalis*, *Myodes glareolus*. The rodents were trapped in urban and rural settings in the high endemic region of Zurich, Switzerland. In each area two study plots were defined, where rodents of the different species were trapped within the presumed range of a single fox territory. In an urban study site significant difference of prevalence rates were found between the investigated species within the same trapping plots during spring 2014: Plot A 65% (95%-CI 50-79), 22% (3-60), 0% (0-15) for *M. arvalis*, *A. scherman*, *M. glareolus*; plot B 47% (24-71), 4% (0-12), 0% (0-9), respectively. In an rural study site the species differences were less pronounced, but significant differences in the prevalence rates were detected with in a distance of less than 500 m: Plot A 2% (95%-CI 2-10), 0% (0-8) and 6% (1-20) for *M. arvalis*, *A. scherman* and *M. glareolus*; plot B 37% (22-54), 22% (9-42) and 0% (0-24), respectively. Interestingly, the overall proportion of individuals with fertile infections was extraordinary high in *M. arvalis* (24%, CI 17-32%) and significantly higher than in *A. scherman* and *M. glareolus* (2% [CI 0-5] and 0% [CI 0-5]). These results give evidence that *M. arvalis*, which is a frequent prey of foxes, is possibly the most important species for the parasite transmission in the region. Furthermore, the study illustrates how the transmission dynamic of *E. multilocularis* can vary considerably within very short distances, even between neighbouring fox territories.
The study of Echinococcus multilocularis focuses predominantly on naturally infected human and animal populations for disease mapping and risk assessment whilst experimental work is often conducted in mouse models intended for medical benefit. Although experimental studies have identified profound differences in the susceptibility of definitive carnivore hosts, limited information exists on experimental infections of this parasite in its naturally occurring intermediate hosts. Such studies would clarify which host species play a key role in transmission, why they are physiologically suited for parasite establishment and growth and which minimum infectious doses would be required in natural settings across various relevant species. In addition to its ecological value, such data would constitute novel information for risk assessment and prevention.

As part of a wider collaborative project investigating the parasite in rodents (EMIRO), various species of intermediate hosts (Microtus arvalis, Microtus agrestis, Myodes glareolus, Mus musculus (CD-1 outbred) and Mesocricetus auratus) were infected with 100 viable E. multilocularis eggs via primary (oral) inoculation. CS7BL/6j inbred mice were used as positive controls for egg viability. Comparison of all species was achieved at 6 weeks post infection (wpi) with further assessment of M. arvalis, CS7BL/6j, M. glareolus and M. auratus at 10 wpi. In addition to the comparison of morphological differences of resulting infections e.g. establishment rate and protoscolex number, the feasibility of measuring immune and endocrine parameters was also investigated. Furthermore, an assessment of varying doses of viable eggs was achieved in M. agrestis indicating possible resource limitation within the host. Results presented here provide baseline biological data on E. multilocularis intermediate host species and thus represent an important contribution to the understanding of E. multilocularis epizootiology.

The fox tapeworm Echinococcus multilocularis is the causative agent of alveolar echinococcosis, a severe disease potentially fatal if untreated. In Western Europe, the red fox (Vulpes vulpes) and small rodents are the main definitive and intermediate host, respectively. Nevertheless, a wide panel of mammalian species, notably aquatic rodents, Suidae or Primates, may be accidentally infected and then considered as aberrant hosts. Many cases of infection by E. multilocularis or other Echinococcus species were reported in captive animals and especially in Primates. In April 2011, liver lesions due to E. multilocularis were observed during necropsy of a captive-born nutria in a French wildlife park, leading to the start of a surveillance study of the presence of the parasite in the park. A similar prevalence of E. multilocularis in fox faeces around (20.6%) and inside the park (17.8%) was observed, as well as the presence of E. multilocularis worms in three of the five roaming foxes shot into the park. Coprological analyses of the captive potential definitive hosts (fox, lynx, wildcat, genet, wolf, bear and raccoon) have resulted to the detection of infection in one gray wolf. A high prevalence of 5.3% in voles trapped inside the park was also reported. After the diagnostic of alveolar echinococcosis in a Lemur catta during necropsy, four other cases in Lemur catta were detected by combination of ultrasound and serology (ELISA and western blot) among the 11 other Lemur catta, four Varecia rubra and three Eulemur rubriventer. The infected lemurs were treated twice daily with albendazole. Despite of approximately two years of treatment, one died and another one has to be euthanized. The systematic massive developmental of metacestode and numerous presence of protoscolex by performing EmsB microsatellite analysis revealing the presence of the same EmsB profiles in surrounding wildlife, but also with a retrospective serological analysis of sera at the arrival to the park. Preventive measures concerning presence of foxes, contact with potential definitive hosts and contaminated food sources for potential intermediate hosts were implemented.
The role of the pericyst – digestive anastomosis in the surgical treatment of the hepatic hydatid cyst

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Introduction: Cystic Echinococcosis (CE) is a zoonotic disease commonly present in regions where animal husbandry is practiced. Romania is considered as one of the most endemic areas for CE in Europe. The most common abdominal location in humans is the liver. The surgical treatment includes conservative methods such as not removing or partially resecting the intraperitoneal pericyst cavity and radical methods, which totally remove the pericyst cavity, with a smaller or larger sacrifice of the liver parenchyma. An important part of the surgical conservative methods is the attitude towards the pericyst remaining cavity. Pericyst – digestive anastomosis is, along with other methods, one of the feasible way to treat the remaining cyst cavity, but under precise indications.

Material and method: In the Surgery Clinic of Colentina Hospital, during 2010–2014, 136 CE patients were hospitalized. Among these, 124 showed strictly intrahepatic location and 12 mixed intra/extrahepatic location. In 9 cases, in which precise preoperative and/or intraoperative indications were identified, we practiced pericyst–digestive anastomosis, as treatment of the residual cavity (6 with the jejunum, using the Roux en Y technique and 3 with the stomach). The postoperative evolution was favorable, except for a case, in which reintervention for solving an abscess developed in the remaining cavity was needed.

Results: Low number of cases in which pericyst-digestive anastomosis were performed was determined by the limitations of this type of intervention to patients with a certain localization of the cyst. The pericyst-jejunal anastomosis was more gainful because it completely excludes the pericyst cavity from the digestive transit.

Conclusions: The aim of the pericyst-digestive Anastomosis is to drain the bile from the remaining cavity in the digestive tract, eliminating the disadvantages of the persistant postoperative biliary drainage from the residual cavity (the obligatory declivity of the fistula or the closure of the orifice of the fistula before the disappearance of the residual cavity).

Keywords: Hydatid disease, pericyst-digestive anastomosis

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Imuno-cytological diagnosis of Echinococcus multilocularis using the monoclonal antibody EM2G11 as compared to more than 100 AE and CE lesions

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Background: Differential diagnosis with cystic echinococcosis (CE) caused by Echinococcus granulosus and alveolar echinococcosis (AE) caused by Echinococcus multilocularis is challenging. Gold standard for diagnosis is histological evaluation on the resected specimen (Barth et al. 2012).

Material and Methods: We present the case of a patient with upper abdominal pain and an unclear retroperitoneal 5 cm measuring mass in projection onto the tail of the pancreas. Data were compared to an established tissue bank including 102 paraffin archived specimens, including 6 cutting needle biopsies and 3 fine needle aspirates, from patients with suspected AE or CE. For analyses we used the monoclonal antibody (mAb) Em2G11 specific for the Em2 antigen of E. multilocularis metacestodes.

Results: In human tissue, staining with mAb Em2G11 is highly specific for E. multilocularis metacestodes, while no staining is detected in CE lesions. In addition, the antibody detects small particles of E. multilocularis (SPEMS) of less than 1 µm outside the main lesion in necrotic tissue, liver sinusoids and lymphatic tissue most probably caused by shedding of parasitic material. The conventional histological diagnosis based on haematoxylin and eosin and PAS stainings were in accordance with the immunohistological diagnosis using mAb Em2G11 in 96 of 102 samples. In 6 samples conventional subtype diagnosis of echinococcosis had to be adjusted when revised by immunohistology with mAb Em2G11.

To test efficacy of the presented technique on small amounts of diagnostic material a fine-needle aspiration of the 55 to 40 mm measuring mass was carried out with a 19-G needle in the presented patient. From the extracted liquid cytospin preparations were performed. Conventional stainings showed disintegrated granulocytes and an abscess was suspected. A Periodic-Schiff staining (PAS) and immune-cytochemical staining with the monoclonal antibody EM2G11 were performed. The staining with the antibody EM2G11 showed strong positive signals of remnants of the lamellar body and multiple positive small particles of Echinococcus multilocularis (SPEMS).

Conclusion: The use of an immune-cytological staining with the EM2G11 antibody on resection specimen and aspiration smears is a cheap, and fast and highly reproducible method to definitely confirm a suspected infection with Echinococcus multilocularis even on low amounts of aspirated material.

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Background: Human alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* is rare, diagnosis and treatment are often a challenge. We report our interdisciplinary approach to all patients referred with AE-diagnosis to the specialized outpatient clinic at the University Hospital Ulm. WHO-PNM classification was the basis for treatment decisions according guidelines of the expert consensus of the WHO Informal Working Group on Echinococcosis (IWGE).

Patients: From January 2012 to July 2015 117 AE-patients were treated at Ulm University. According to the WHO case definition AE was “confirmed” in 71 and “probable” in 41 patients. 5 “possible cases” were excluded. 65 were female (58%) and 47 male (42%), median age at first diagnosis was 51 years (range 11 to 82).

2 patients presented with stage I, 35 with stage II and 15 with stage IIIA, meaning a good chance for complete resection of the AE-lesion.

Over 50% presented in advanced stages: 33 patients IIIB (extrahepatic involvement (N1) or P4 lesion (extension along vessels and biliary tree); 27 presented with stage IV, (P4 lesion + extrahepatic involvement or presence of distant metastasis (M1), meaning cure is unlikely for those patients.

Treatment: In patients with “probable” or “confirmed” AE benzimidazole (BMZ) treatment was generally advised (n=110), but for 2 we suggested watch and wait. In advanced AE the albendazole (ABZ) treatment was advised to be long-term, whereas after complete resection BMZ for 2 years were recommended. 45 patients underwent surgery, i.e. 34 in curative intent (safety margin > 1 cm; additional 7 patients had a complete removal of their lesions with a safety margin < 1 cm). 4 patients had a palliative resection, reasons were haemorrhage, pain and also lack of knowledge regarding the therapy recommendations. BMZ long-term therapy is recommended for those.

14 patients had ABZ intolerance and 8 did not tolerate BMZ at all. All 112 patients are alive. 35 of 45 patients after surgery are regarded as cured.

Conclusion: Staging (PNM classification) is condition for a structured therapeutic approach to AE. All patients should receive benzimidazoles, with very few exceptions. Only around 30% of AE-patients can be cured (PNM stage I to III a). For most patients AE is a chronic disease with the need for long-term drug therapy. As AE is rare, expertise is best acquired in a specialized institution; a benefit for the patients results from adherence to the WHO treatment recommendations.

Pitfalls in diagnosis and treatment of *alveolar echinococcosis*: a sentinel case series

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Background: Alveolar echinococcosis (AE) is a neglected zoonosis presenting with focal liver lesions (FLL) with a wide range of imaging patterns resembling benign as well as malignant FLLs.

Complementary serology and histopathology may be misleading.

Objective: The objective of our study is to highlight pitfalls leading to wrong diagnoses and harmful interventions in patients with AE.

Design: This retrospective sentinel case series analyses diagnostic and treatment data of patients with confirmed AE.

Results: 80 patients treated between 1999 and 2014 were included in the study. In 26/80 patients’ treatment decisions were based on a wrong diagnosis. AE was mistaken for cystic echinococcosis (CE) in 12/26 patients followed by cholangiocellular carcinoma (CCA) in 5/26 patients; 61/80 patients had predominantly infiltrative liver lesions and 19/80 patients had a predominantly pseudocystic radiological presentation. Serology correctly differentiated between *Echinococcus multilocularis* and *Echinococcus granulosus* in 53/80 patients. Histopathology reports attributed the right Echinococcus species in 25/58 patients but failed to differentiate *E. multilocularis* from *E. granulosus* in 25/58 patients. Although contraindicated in AE 8/25 patients treated surgically had instillation of a protoscolicidal agent intraoperatively. One of the eight patients developed toxic cholangitis and liver failure and died 1 year after liver transplantation.

Conclusions: Misclassification of AE leads to a critical delay in growth inhibiting benzimidazole treatment, surgical overtreatment and bares the risk of liver failure if protoscolicidal agents are instilled in AE pseudocysts.


ORAL PRESENTATIONS
Analysis of classification and imaging features in children with hepatic alveolar echinococcosis

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Objective: To observe the imaging features of CT and MRI in hepatic alveolar echinococcosis of children and explore its classification and PNM staging.

Methods: 31 patients aged 2-14 years (15 boys, 16 girls, average age: 9.4 years old) with HAE were examined using CT and MRI examination. Imaging analyzed including the lesion's location, size and blood supply, the peripheral zone and imaging type of lesions were assessed and recorded based on invasion and metastases in order to clarify its PNM staging.

Results: The period with disease of 31 patients was 0.6-3 years and the average was 1.25 years. All patients showed moderate to severe malnutrition, 23 cases of abdominal pain and jaundice, 17 cases of varying degrees of liver function damage. There were 43 hepatic lesions in 31 patients, of which 2 cases the lesion invaded the whole liver. The lesion location: a total of 11 cases (35.5%) of the left and right lobe, 6 cases of both the left and right lobe (19.35%), 9 cases on the right lobe (29.03%), 3 case the left hepatic lobe, 6 cases of both the left and right lobe (19.35%), 8 cases of massive type and the liquefaction necrosis type were major classification. The lesion invaded the branches of portal and hepatic veins mainly with venous bloodsucking growth characteristics metastasis-prone, PNM advanced stage.

Conclusion: The lesions of HAE in children growth rapidly with high activity peripheral zone on DWI. The massive type and the liquefaction necrosis type were major classification. The lesion invaded the branches of portal and hepatic veins mainly with venous bloodsucking growth characteristics metastasis-prone, PNM advanced stage.

Keywords: children, hepatic alveolar echinococcosis, CT and MRI, classification

Ex vivo liver resection and autotransplantation for end-stage Alveolar Echinococcosis: a case series

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The role of autotransplantation in end-stage hepatic alveolar echinococcosis (HAE) is unclear. We aimed to present our fifteen cases’ experience and propose selection criteria for autotransplantation. All patients were considered to have unresectable HAE by conventional resection due to critical invasion to retrohepatic vena cava, hepatocaval region along with three hepatic veins and the tertiary portal and arterial branches. All patients successfully underwent ex vivo extended right hepatectomy and autotransplantation without intra-operative mortality. The median autograft weight was 706 g (380 - 1000g); operative time 15.5 hours (11.5 - 20.5 hours); anhepatic time 283.8 minutes (180 - 435 min). Postoperative hospital stay was 32.3 days (12 - 60 days). Post-operative complications (58%) of Hepatic vein and 8 cases (25.81%) of inferior vena cava invasion. 23 cases (74.19%) of bile duct invasion. The peripheral zone of DWI showed high signal intensity, ADC value were 0.877±0.137×10-4 mm2/s. Metastases: 13 patients of 31 cases had metastasis (41.9%), where in massive type with 9 cases (69.2%) and liquefaction necrosis in 4 cases (30.8%). The main sites of metastasis included lung metastasis, brain metastases and vertebral metastases.

PNM stage: 15 cases of P4 stage (48.39%), 6 cases of P3 stage (19.35%), 8 cases of P2 stage (25.81%); 2 cases of P1 stage (6.45%); 8 cases of N1 stage (25.8%), 23 cases of N0 stage (74.2%); 13 cases of M1 stage (41.94%), 18 cases of M0 stage (58.06%).

Conclusion: The lesions of HAE in children growth rapidly with high activity peripheral zone on DWI. The massive type and the liquefaction necrosis type were major classification. The lesion invaded the branches of portal and hepatic veins mainly with venous bloodsucking growth characteristics metastasis-prone, PNM advanced stage.

Keywords: alveolar echinococcosis, ex vivo resection, liver autotransplantation, regeneration
Immunomodulation of host immunity by larval Echinococcus multilocularis

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The metacestode stage of Echinococcus multilocularis that causes alveolar echinococcosis (AE) consists of a cluster of tightly mingled fluid-filled vesicles. The outer acellular surface of these vesicles is formed by the laminated layer, a carbohydrate-rich structure synthesized by the parasite. The laminated layer plays a crucial role in the survival strategy of the parasite by modulating immunological and physiological reactions on part of the host. The E. multilocularis metacestode reproduces asexually, by exogenous formation and budding of daughter vesicles, which overall resembles progressive tumour-like growth. Metastases formation may occur in other organs due to release of parasite micro-vesicles into the blood or lymph system. While growing and proliferating, the metacestode induces a strong periparasitic cellular immune reaction. The type/nature of this reaction turns out to be crucial with regard to either control or fail to control parasite proliferation and thus disease. T cell-deficient mice as well as HIV-co-infected patients exhibit high susceptibility to infection and disease, herewith suggesting that the host cell mediated immune response plays an important role in suppressing the larval growth. In immunocompetent but susceptible hosts, a progressive impairment of MØ and DC maturation and antigen presentation allows a continuous E. multilocularis proliferation. Subsequent experiments at the T cell level documented that regulatory T cells (Tregs) interfere in the complex immunological host response to infection. Our most recent data demonstrated now that a novel CD4+CD25+ Treg effector molecule FGL2 contributes to the outcome of E. multilocularis infection by promoting Treg cell functions (Wang et al., 2015); they give evidence for a role of IL-17 in FGL2 regulation and suggest that targeting FGL2 could be used for the development of novel treatment approaches in this parasitic disease.

The lesion growing characteristics of hepatic alveolar echinococcosis: DCE CT and MRI findings

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Aim: To study the growth characteristics and the lesion’s activity of hepatic alveolar echinococcosis (HAE) by dynamic contrast enhanced (DCE) CT and MRI.

Methods: The patients suffered from HAE were enrolled consecutively from September 2013 to June 2015 and dynamic contrast enhanced (DCE) CT and MRI scan were obtained from 102 cases. There were 102 cases of HAE which diameter was ≥50mm. We observed the type, size and the direction of the invasive growth, the characteristics of the arteriovenous blood supply and the manifestation of the lesions of all cases. The diagnosis was based on 3D reconstruction images and combined with 2D images. The growth direction and the range of the edge zone were defined by DCE and DWI.

Results: 102 cases of patients with intrahepatic lesions (diameter was ≥50mm) were 127. There were 51 cases (40.16%) located in the right lobe of the liver, 20 cases (15.75%) left lobe of the liver and 56 cases (44.09%) invade at and left lobar of liver. The growth characteristics of hepatic lesions were the growth direction to the blood vessel-rich region. The first and second hepatic portal was particularly encroached. If there had no blood supply, the liver edge basically stopped growing and the edge might collapse. Portal vein was the widest invasion region, including the trunk and left and right branch vessels. There were a total of 113 lesions (89%). There were 74 lesions (65.5%) which the first hepatic portal were violated and followed by a total of 41 cases (36.3 %) which the hepatic vein and inferior vena cava were encroached. The edge zone of the lesions on DWI was high signal, which was located in the center of the active direction of the growth and enhanced in venous phase. There were 48 cases (47.1%) of 102 cases with other organs metastases. The main sites of metastasis included double lung metastasis, brain metastasis, vertebral metastasis, retroperitoneal and so on.

Conclusion: The growth characteristics of the solid type of HAE were mainly invasive growth to the rich vascularity of hepatic hilar region. The lesions mainly invade in portal and hepatic vein, which are addicted to vein blood, so they are easy to metastasize. The HAE’s peripheral zone can be enhanced in the venous phase, which we named “active growth zone”.

Keywords: HAE, DCE-CT and MRI, growth, active growth zone
Immunotherapy in secondary murine alveolar echinococcosis: assessment of the recombinant Echinococcus multilocularis EmP29 (rEmP29)

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Human alveolar echinococcosis (AE) is a life-threatening infection caused by the larval stage of Echinococcus multilocularis (E. multilocularis). Current medical management of AE that relies on surgery and continuous benzimidazole administration is of limited effectiveness. Immunotherapy for cancer treatment has shown promise in early clinical trials; however, its potential for the treatment of infectious diseases remains to be investigated.

We assessed for the first time, the immunotherapeutic potential of the recombinantly expressed E. multilocularis P29 (rEmP29) antigen for treatment of secondary AE in mice. Experimentally infected mice were treated with the rEmP29 vaccine, starting at 1 month post-infection, three times with 2 weeks intervals. Mice undergoing rEmP29 immunotherapy exhibited a median parasite load that was reduced by 53% when compared to nontreated mice.

Results from the implementation of the National programme for control of echinococcosis in humans and animals in Bulgaria from 2004 to 2008

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In 1998, in Bulgaria, was registered a dramatically increase in the incidence of hydatid disease in humans (8.47 per 100,000 population) in comparison with the low levels in the 80s of the 20th century (1.75 to 3.1 per 100,000 population). These figures resulted in the implementation of National programme for control of echinococcosis in humans and animals for the period 2004 - 2008. The aim of the programme was to establish a lasting trend of reduced infection, a sharp decrease of morbidity among humans and animals and the following economic and medico-social damage.

Unfortunately only the medical part of the programme was completed because of insufficient funding for the Ministry of Agriculture and Food. As a result from the implementation of the programme national and regional database of echinococcosis in humans was established. Morbidity of 712 villages and towns throughout the country was registered in the database. During the period from 2004 to 2008, 2,867 persons with hydatid disease were registered and in all cases specially designed epidemiological carts were filled. 28,485 persons were tested serologically for echinococcosis. 19,095 were tested by referring and 9,390 were included in immunoscreening. A total of 551 were the newfound cases and after conducting an image diagnostics and treatment, the diagnosis for echinococcosis was confirmed histologically in 66 individuals without clinical symptoms. One of the main tasks of the programme was to perform mass immunoscreening for early detection of hydatid disease among risk groups in 9 regions of the country - Burgas, Varna, Pleven, Plovdiv, Sliven, Smolyan, Stara Zagora, Haskovo and Yambol. A total of 9,390 individuals from these regions were studied, divided into the following groups: children and adolescents up to 19 years (1,426), family members of the diseased (348), persons at risk (2,315) and individuals studied by their request (5,231). Positive serological results were obtained in 107 tested individuals. During the programme new regulations were developed that led to the improvement of the registration and notification of the diseased.
Echinococcus granulosus sensu lato species complex is the causative agent of cystic echinococcosis, a zoonotic disease of worldwide importance and global distribution. The parasite E. equinus, formerly known as the horse strain (G4) of E. granulosus, is firmly established as an independent species according to the second taxonomic revision. A 2-year-old female donkey (Equus asinus) was euthanized in the Pathology Department of Firat University, Elazig, Turkey. Necropsy revealed the presence of seven fluid-filled hydatid cysts distributed throughout the lung parenchyma. Microscopic examination of wet unstained preparations revealed the presence of abundant protoscoleces in all the seven cysts. A 3cm in diameter hydatid cyst was isolated from the left cranial lobe of the lung and represented the parasite material of the present study. The cyst was molecularly identified through sequencing of a fragment of cytochrome oxidase subunit 1 (CO1) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (NADH1) gene, as Echinococcus equinus. The molecular identification of E. equinus in a donkey is being reported for the first time in Turkey.

**Expression of Toll-like receptor 2, 4 and related cytokines in patients with hepatic cystic and alveolar echinococcosis**

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Several studies have demonstrated the important role of Toll like receptors in various parasitic infections. This study aims to explore expression of Toll-like receptors (TLRs) and related cytokines in patients with human cystic (CE) and alveolar echinococcosis (AE). 78 subjects including AE group (N=28), CE group (N=22) and healthy controls (HC, N=28) were enrolled in this study. The mRNA expression levels of TLR2, TLR4 in blood and hepatic tissue and plasma levels related cytokines were detected by using ELISA. Median levels of TLR2 mRNA in AE and CE groups were significantly elevated as compared with that in healthy control group. Median levels of TLR4 expression was increased in AE and CE. Plasma concentration levels of IL-5, IL-6 and IL-10 were slightly increased in AE and CE groups than those in HC group with no statistical differences (p>0.05). The IL-23 concentration levels were significantly higher in AE and CE groups than that in HC subjects with statistical significance. The Expression pattern of TLR 2 and 4 in PBMCs in patients with AE and CE might be involved in the cytokine modulation, which allowed the parasite to escape, which seems to be stronger in AE, host immune-surveillance and promoted chronic infection.

**Deep proteomic analysis of hydatid cyst fluid of Echinococcus granulosus and E. multilocularis**

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**Background and aims:** The two major species of Echinococcus – E. granulosus and E. multilocularis - cause cystic echinococcosis (CE) and alveolar echinococcosis (AE), respectively. One significant difference between the parasites is that E. multilocularis causes a serve pathological response. However, the mechanisms underlining the difference are not clear. The differential proteins secreted by E. multilocularis are believed the key elements causing the difference. The aim of the present study is to reveal the difference of protein profile in hydatid cyst fluid (HCF) of the two close parasites.

**Methodology/principal findings:** We have successfully cultured protoscoleces of the two parasites, which develop to hydatid cysts in an exactly the same culture condition. We then collected hydatid fluid from the cultured cysts and subjected to a liquid chromatography-tandem mass spectrometry (LC/MS/MS) proteomic analysis to define the protein profiles in the two sources of HCF. Totally, we identified 774 proteins in E. granulosus HCF, 2305 proteins in E. multilocularis HCF with 39 bovine serum proteins. Among them, the classical secretary proteins of E. granulosus and E. multilocularis were 52 and 122 respectively. Gene Ontology analysis revealed that the proteins involved in cellular process, metabolic process, response to stimulus, biological regulation, binding catalytic activity were highly existed in the hydatid fluid of E. granulosus and E. multilocularis.

**Conclusion:** The differential proteins may play a key role in causing the pathological difference between the two parasites in the intermediate hosts.

**Keywords:** Echinococcus granulosus, E. multilocularis, hydatid cyst fluid, proteomics, secretary protein
Real-time loop-mediated isothermal amplification assay for simple and rapid detection of cystic echinococcosis, Sudan

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Background: Cystic echinococcosis (CE) or hydatidosis, caused by the larval stage of Echinococcus granulosus (EG)-complex, is a neglected parasitic disease of public health importance. The disease is endemic in many African and Mediterranean countries including the Sudan. A simple and rapid molecular-based assay is urgently needed for diagnosis of the disease. The objective of the present study was to develop and evaluate a real-time loop-mediated isothermal amplification (LAMP) assay for simple and rapid detection of CE in humans and domestic live stock in Sudan.

Methods: A set of six LAMP primers, designed from the mitochondrial NADH-1 gene of EG cattle strain of genotype 5 (G5), was used as a target for LAMP assay. The assay was performed at a constant temperature (63°C), with a real-time follow-up using a LightCycler and fluorochrome dye. Following amplification cycles in a simple water bath, LAMP products were observed for color change by naked eye and were visualized under UV light source using agarose gel electrophoresis.

Results: The real-time LAMP assay identified a variety of hydatid cysts strains recovered in the Sudan, including Echinococcus canadensis (G6) and Echinococcus ortleppi (G5). Real-time LAMP positive results were detected by the presence of an amplification curve, whereas negative results were indicated by no fluorescence. Positive LAMP results appeared as a bluish-colored reaction as observed by naked eye, whereas negative LAMP results were observed as purple-colored reaction. The sensitivity studies indicated that the LAMP assay detected as little as a 10 fg of parasite DNA. There was 100% agreement between results of the LAMP assay and our previously described nested PCR when testing 10-fold serial dilution of DNA extracted from EG-complex hydatid cyst. However, there was no cross-reactivity with other parasites including Cysticercus bovis, Fasciola gigantica, and Schistosoma bovis and nucleic acid free samples. The LAMP assay provides high levels of diagnostic sensitivity and specificity when testing a variety of archived hydatid cyst specimens, from humans and domestic livestock, on a practical scale.

Conclusion: The developed LAMP assay would be expected to prove highly significant in epidemiological surveys of CE in developing countries or areas of resource-poor settings. The specific PCR product generated by the outer pair of LAMP primers (F3 and B3) could be employed for sequencing and subsequent genotyping of the hydatid cyst strain.

Strain characterization of E. granulosus bovine cyst and serological interleukin levels in hydatidosis and distomatosis infected cattle

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Introduction: Hydatidosis (HT) is a recognized public health problem in Chile, although molecular information regarding strains and prevalence of hydatid cyst (HC) are still lacking. Genotypes of the parasite (G1-G10) are known to exist and this study is aimed to identify Echinococcus granulosus in bovine HC, to evaluate the similarity of the genotypes between organs and to characterize serum interleukin concentration in HT and DS infected cattle.

Materials and Methods: A cross-sectional study was conducted on 2,781 animals to characterize the presence of HT and DS in cattle slaughtered at an abattoir in Santiago, Chile. Postmortem examination comprised visual inspection and incision of lungs and liver for the presence of HC and liver fluke. 256 HC were obtained from bovine lungs (192) or livers (64) for molecular characterization of E. granulosus. Protoscoleces or germinal layers were collected from individual cysts, DNA extracted following a commercial kit (Promega) and part of the mitochondrial DNA encoding cox1 gene was amplified by Alul PCR-RFLP. Also, 10 serum samples were obtained for the standardization of a commercial MULTIPLEX kit (EMD Millipore) to determine IL-4 and IFN-γ concentration.

Results: Overall prevalence of HT was 18% (503/2781), DS 13% (373/2781) and animals with DS and HT together 3% (75/2781). Out of 503 cattle harboring one or more HC in their internal organs, 54% (271/503) had cysts only in their lungs and 19% (97/503) only in their livers. The rest of the infected cattle (27%) had concurrent infection in livers and lungs (135/503). From all the recovered cysts: 186 (97%) from lungs and 70 (98%) from liver corresponded to G1 strain, while other strains (G2-G10) were scarce (3%). IL-4 concentration in HT infected animals (51.05±28.87ng/mL) was significantly lower (p<0.05) compared to bovines co-infected with HT and DS (230±15.55 ng/mL), while IFN-γ concentration was under the detection limit of the kit. Discussion: Hydatidosis and distomatosis prevalence in cattle is in accordance with previous reports from Chile. Molecular characterization of hydatid cysts confirmed the dominance of G1 strain in Chile as it is in the rest of the world, and showed no difference in the genotypification of E. granulosus between liver and lung cysts. In addition, serum analysis suggests that co-infection with distomatosis would be a factor that affects IL-4 concentration in hydatidosis infected bovines. Grant from FONDECYT/Chile No. 1130717.
Evaluation of chemotherapy in treating ewes naturally infected with hydatid disease

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Aim: Chemotherapy is an effective method to treat hydatid disease, such as albendazole, which can be widely used for treatment of human and animals infected with E.g. Same as human, sheep is intermediate host of E.g. Sheep infected with E.g. always treated with Chemotherapy. Liposomal albendazole (L-ABZ) is a nanometer new form of medicine for treating hydatid disease, showed good clinical effective to hydatidosis. In our study, a randomized blindly controlled trial was designed to compare the effect of L-ABZ and emulsion albendazole (E-ABZ) and we tried to evaluate the effect of L-ABZ and E-ABZ in treating hepatic hydatid disease of ewes.

Material and Methods: 30 ewes naturally infected with hydatid disease were selected to assign three groups randomized, treated with L-ABZ (group A, n=10), E-ABZ (group B, n=10) and placebo (group C, n=10) blindly, after continuous chemotherapy for three months, Ultrasound(US) and CT scan were used to evaluate the effect of the three groups.

Results: After all groups were unblinding, in the course of continuous chemotherapy, 5 ewes had died in group taking E-ABZ, all ewes survived in groups taking L-ABZ and placebo. After checked by ultrasound and CT scan for liver of ewes in three-months-chemotherapy, general effective rate showed difference. SPSS17.0 statistics software was used to analyze the results. Statistical data showed there are differences between group A and group B based on US (Z=-2.983, P=0.003) and CT scan (Z=-3.297, P=0.001), group A is prior to group B. There are no differences between group B and group C based on US (Z=-0.573, P=0.567) and CT scan (Z=-0.573, P=0.567), group B is not prior to group C.

Conclusion: L-ABZ showed good effect in treating hepatic hydatid disease for naturally infected ewes, E-ABZ showed limited effect in this study, the effect of L-ABZ is obviously prior to E-ABZ. Furthermore, it is noteworthy that it is possible to have serious side-effect for E-ABZ in treating ewes naturally infected with hydatid disease. On the country, L-ABZ showed more safety than E-ABZ.

Keywords: hydatid disease, liver, chemotherapy, effect

Intra-cystic drug concentration of albendazole and its active metabolite albendazole-sulphoxide in human cystic echinococcosis: a systematic review

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Aim: Albendazole - the first-line anthelminthic drug for medical treatment of cystic echinococcosis - is metabolized in vivo to the active derivative albendazole-sulphoxide (ABZ-SO). Target site ABZ-SO concentrations in the echinococcal cyst mediate the anthelmintic effect in human cystic echinococcosis. We present a systematic review of current evidence on intra-cystic target site concentrations of ABZ-SO.

Methods: A systematic review and pooled analysis of individual patient data was performed from all available published reports. The main outcome of this systematic review was the description of target site concentrations of ABZ-SO and potential co-variables.

Results: Pharmacokinetic data of 121 individual cysts were analyzed. There was no correlation between ABZ-SO concentrations in plasma (median 240µg/L; 25th-75th percentile: 135 - 538µg/L) and cyst fluid (median 198µg/L; 25th-75th percentile: 94 - 449µg/L; rho=-0.05, p=0.58). Intra-cystic drug concentrations were not associated with gender or treatment duration. The use of praziquantel in combination with ABZ was associated with higher ABZ-SO concentrations in plasma (540µg/L (25th – 75th percentile: 255 - 1020µg/L) and 240µg/L (25th – 75th percentile: 132 - 535µg/L), p=0.03) but not in cysts (220µg/L (25th – 75th percentile: 170 - 510µg/L) and 198µg/L (25th – 75th percentile: 92 – 417µg/L), p=0.37). Target site concentrations were higher in calcified than non-calcified cysts (median 897µg/L; 25th to 75th percentile: 504 - 2763µg/L compared to 84µg/L; 25th to 75th percentile: 32 – 368µg/L; p=0.09). Cyst concentrations were lower in large sized cysts (β=-17.2µg/L, 95th CI: -35.9 - 1.6; p=0.07).

Conclusion: This systematic review demonstrates that intra-cystic drug concentrations are similar to plasma concentrations. However, blood concentrations are not directly predictive for cyst concentrations. Intra-cystic concentrations are higher in calcified and smaller cysts. The use of booster drugs was not associated with higher intra-cystic ABZ-SO concentrations in this review.
**Surgical and molecular evaluation of pediatric hydatid cyst cases and some risk factors in Eastern Turkey**

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The aims of this study were to evaluate the strain(s) and risk factors of *E. granulosus* in operated pediatric cases in eastern Turkey. Ten pediatric patients, ages ranging from 3 to 15, with various complaints were included in this study. The cases, whose cyst hydatid haemagglutination tests were positive, were diagnosed with ultrasonography and computerized tomography and diagnoses were confirmed by the surgery. The patients consisted of 7 boys and 3 girls, eight patients had only liver hydatid cyst while two patients had multiple cysts of various organs. During follow-up, no increase was observed in haemagglutination levels and there was not any mortality and recurrence in all patients. Molecular analysis was performed on hydatid cyst samples obtained from 10 pediatric cases residing in 4 different provinces in eastern Turkey. According to mt-12S rRNA PCR results, all cases were found to be G1/G3 cluster (*E. granulosus sensu stricto*). A conservative surgical technique is sufficient in most cases. We can conclude that common sheep strain may be more possible for pediatric cases due to the domestic cycle. Besides some risk factors such as poor hygiene condition, contact with dog and uncontrolled livestock slaughtering should be consider and patients should be informed about these.

**Giant hydatic hepatic cysts – trying to hill better and to harm less**

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**Background:** Giant hydatic hepatic cysts are not very common in the surgical practice. We defined as giant cysts those exceeding 15cm in diameter. For these hydatic cysts the biliary fistulae should be presumed in postoperative evolution, but they could already exist in the moment of surgery. An appropriate surgical approach should be performed according to their placement.

**Material and methods:** During 01.2013-07.2015, 98 patients with hydatic hepatic cysts were operated in Colentina General Surgical Clinic. 31 patients (31.63%) had hydatic cysts with large dimensions (over 10 cm), and furthermore, 6 (6.12%) patients have had giant hepatic cysts, all of them developed in the right hepatic lobe. These are the target of our study.

The surgical approach was Lagrot operation (partial pericystectomy, cyst removal, drainage of the restant cavity) in 5 cases. The external biliary drainage was used in 3 of these cases. One case was solved using pericyst-digestive anastomosis, but the external biliary drainage was used, too. ERCP was required in postoperative care to decrease the biliary draining flow in 5 cases.

**Results:** Finally, the evolution was good in all cases. Hospitalization was between 38-116 days, but it is important to remark the usefulness of the external biliary drainage, which shortened the hospitalization (none over 60 days). Without such approach, 91 and 116 days were needed for these patients.

**Conclusions:** Lagrot operation remains an important option in the giant hydatic cysts of the posterior side of the right hepatic lobe; giant hydatic cysts of the anterior side and visceral surface of the liver allow a pericyst-digestive anastomosis. ERCP is a good solution for the persistent biliary drainage, which is seen almost in every case; external biliary drainage is an important tool to reduce the biliary leakage. Surgery for this kind of hydatic hepatic disease requires well trained teams which could perform various procedures. Diagnosis and monitoring of the cases should be done in specialized centres, by a multidisciplinary team.

**Keywords:** giant hydatic hepatic cyst, biliary fistula, Lagrot operation, pericyst-digestive anastomosis, external biliary drainage, ERCP

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Quality of medical care is built upon years of effort by scientists and medical professionals investigating and researching the causes of and potential treatments for disease. Research in developing countries has been lagging behind, a problem which has resulted in the slow reduction of diseases. Developing countries urgently need research to help relieve the burden of disease and to increase knowledge on ways of prevention.

Al Neelain University has taken the lead to improve researches at the university level through implementing a system for the conduction of research at the undergraduate level, with the vision and aim that it will make undergraduates get exposed to research and make research practice a common culture among the newly graduated doctors.

The research methodology course is taught in the third year and a final project is presented at the end of the fourth year. Problems with the research methodology course were reduced student's interest; supervisors did not have enough knowledge and skill to supervise students. Other challenges were research funding and supervisors' work load.

**Aim:** Improve the quality of undergraduate researches through implementation of a strong system for the conduction of research.

**Materials and methods:** A longitudinal study was performed. Participants in the course were students from the 3rd year. The year had 122 students; all were enrolled in the study. The follow-up took another two semesters. The system implemented was to concentrate on the following main domains: research methodology course teaching; standardization of the examination system; training of supervisors; incentives for research.

**Results:** Attendance rates improved, where 96% attended more than 75% of the course. 86% submitted a proposal at the end of the 3rd year; the rest submitted it in year four. All of them submitted the final thesis. 75% scored A and B grades, while the rest scored C and some of them D grades. 2% of the students published their thesis, 65% of them are thinking of publishing.

**Conclusion:** Research practice has improved; further follow-up will be done to further increase the quality as well as the publication of researches at the university.

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New treatment options against alveolar echinococcosis (AE) are urgently needed since the currently used benzimidazole treatment has several drawbacks. Previous studies have shown that anti-malarial compounds such as mefloquine and artemisinins exhibit good *in vitro* (and *in vivo*) activities against *E. multilocularis*. We therefore further pursued investigations on the activities of anti-malarial drugs and screened the MMV (Medicine for Malaria Venture) malaria-box, a drug candidate library consisting of 400 compounds with *in vitro* activity against *Plasmodium falciparum*. The screening was based on an extended screening cascade including (i) the previously described *in vitro* PGI metacestode damage assay, (ii) *in vitro* host cell toxicity assays, (iii) *in vitro* germinal layer cell toxicity assays and (iv) an *in vitro* metacestode viability assay. Through this cascade, several compounds with anti-metacestode activities were identified, and one compound, the salicylanilide MMV665807, showed a potential therapeutic window and exhibited parasiticidal activity *in vitro*. *E. multilocularis* calmodulin was identified as a direct binding partner of MMV665807. Recombinantly expressed EmCalmodulin was functional in an enzymatic assay and was directly inhibited by MMV665807. RNAi-mediated inhibition of EmCalmodulin expression demonstrated loss of viability of germinal layer cells. MMV665807 was also tested for its effects on parasite load in a murine AE model, both by p.o. and i.p. application at 100 mg/kg. However, *in vivo* MMV665807 did so far not have any effect on parasite growth. MMV665807 is structurally related to niclosamide, and further investigations will focus on other related compounds with improved bioavailability and pharmacokinetic properties.
Application of individualized therapy in surgical treatment of hepatic hydatid disease and its clinical significance

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Objective: To discuss the necessity and clinical significance of individualized treatment for liver cystic echinococcosis.

Methods: Retrospective analyze the data of 249 cases of patients with hepatic cystic echinococcosis (HCE) which underwent individualized surgical treatment from July 2011 to January 2015 and 223 cases of patients with hepatic cystic echinococcosis (HCE) which underwent experiential surgical treatment from December 2007 to January 2011 in the first people’s hospital of Kashi, so as to make further comparison between the two groups at the average postoperative hospitalization days, the average postoperative intubation time, postoperative residual cavity complications (bile duct leakage, infection, effusion etc.), postoperative liver function, peritoneal dissemination, hydatid cyst recurrence in situ, death rate etc. and evaluate the clinical effects and significances of the individual treatment. Operation methods including: Improved endocystectomy, Total pericystectomy, improved endocystectomy + subtotal pericystectomy and Partial hepatectomy. According to the HCE’s standard size, location, nature, whether there are complications, relationships with peripheral vascular and organizations and then take the patient condition and other factors in to consideration, combined with clinical technology and experiences from previous treatments for HCE, select the best optimal individualized surgical operation for the 249 cases of HCE patients. According to different operative methods the 249 cases were divided into four groups: Improved endocystectomy group (group A) were 55 cases, Total pericystectomy group (group B) were 102 cases, Improved endocystectomy + subtotal pericystectomy group (group C) were 73 cases, Partial hepatectomy group (group D) were 19 cases. All data were analyzed by using the t test and the chi-square test.

Results: The cure rate of 249 patients which underwent individualized treatment in accordance with the individualized treatment plan was up to 100%, no death case. Compare the two groups’ curative effect after operation, individualized surgical treatment group was better than the experiential surgical treatment group, the differences between the two groups’ therapeutic effect index at, average postoperative hospitalization days, the average postoperative intubation time, postoperative residual cavity complications, postoperative liver dysfunction, has statistical significance (P<0.05). Conclusion: In the surgical treatment of hepatic cystic echinococcosis, the definite diagnosis before operation, combined with the patients’ specific circumstances, through the combination of multiple subjects, correct selection and implementation of operation scheme can improve the cure rate, reduce the operation rate and mortality rate, save medical expenses. Under the guidance of the principle of evidence-based medicine, implement scientific individualized treatment programs for HCE is the ideal choice, is the key to determining prognosis.

Keywords: hepatic hydatid disease, individualized treatment, surgical treatment

Comparison of postoperative short-term and long-term outcomes between different surgical procedures after frank biliary rupture of hydatid disease

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Purpose: The purpose of this study was to evaluate postoperative short term and long-term outcomes in intractable rupture of hydatid disease with partial pericystectomy and T-tube decompression, focusing on the relationship between operative procedures and outcomes.

Method: Between 2000 and 2012 a total of 63 patients underwent partial pericystectomy for frank intrabiliary rupture. Follow-up results were obtained from 51 patients. Their mean age was 47 years (range 29–66). These patients were divided into two groups based on their operative procedures: group A (2000–2006) 28 patients partial pericystectomy with only single T-tube decompression and group B (2006–2012) comprising 23 patients who underwent subtotal pericystectomy with double T-tube decompression with sustained T-tube and decompression T-tube. Biliary orifices seen in the residual cavity were suture with absorbable sutures. We evaluated the short-term and long-term outcomes in the two groups.

Result: Overall complications were seen in total of 14 patients (27.45%). In group A, 10 patients (35.71%) had complications, which included minor biliary fistula in 3, major biliary fistula in 1, wound infection in 1, while one patient developed postoperative abscess. In long term complication in group A 3 had biliary stricture while one patient with recurrence. In group B, none of the patients developed long-term complications, however a total of 4 patients (17.39%) developed short term complications which included 2 minor biliary fistulas, 2 wound infection while one patient developed postoperative abscess.

Conclusions: Although a longer follow-up period is necessary, late complications strictures were more frequent in group A than in group B patients. We believe that in subtotal pericystectomy with T-tube decompression a sustained T-tube is essential for the prevention of postoperative cavity infections and biliary strictures.
Background: *Echinococcus granulosus* is a cestode whose larval stage causes cystic Echinococcosis (CE) in humans. CE is acquired by ingesting eggs, originating from the faeces of definitive hosts (dogs, wolves, and other carnivores), that go through the stomach and harbor the adult *E. granulosus* worms in their small intestine. *Helicobacter pylori* infect gastric mucosa cell in general. The aim of this study is to investigate the relationship of *Echinococcus* and *Helicobacter pylori*.

Methods: ELISA approach was used to detect *Helicobacter pylori* antibody from serum in 13 CE patients (experimental group) and 6 patients without CE (control group).

Results: In experimental group, the positive rate of *Helicobacter pylori* antibody was 84.6%. In control group, the positive rate of *Helicobacter pylori* antibody was 66.7%. There was significant difference (p<0.05).

Conclusions: *Echinococcus* and *Helicobacter pylori* may play a role in the pathogenic effect each other.

Keywords: Echinococcus, Helicobacter pylori

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Cystic echinococcosis in HIV infected patients

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Introduction: Various opportunistic infections have been reported in patients with human immunodeficiency virus (HIV) infection. The most common parasitic infections in HIV infected patients are protozoans. Infections by helminths have been described less often. The most common helminths causing diseases in HIV infected patients are nematodes, especially *Strongyloides stercoralis* or *Strongyloides stercoralis* or *Strongyloides stercoralis* or *Strongyloides stercoralis* or *Strongyloides stercoralis* or *Strongyloides stercoralis* or *Str* _strongyloides stercoralis* and *trematodes* (*Schistosoma* spp). Parasitic infections by cestodes are unusual and are reported especially in endemic regions.

Objective: We evaluated the therapeutic response to therapy with albendazole in HIV infected patients with hydatid cysts.

Methods: We analyzed clinical, biological, serological, imaging and in terms of efficiency albendazole therapy, the patients with cystic echinococcosis and HIV infection diagnosed in HIV/AIDS Constanta Regional Center.

Results: Of the 998 HIV infected patients at the Regional HIV/AIDS Center of Constanța, Romania, we identified only six patients with cystic echinococcosis (P=0.6%, comparing with more than 10 cases/100,000 inhabitants in general population), five patient with liver hydatid cysts, one patient with peritoneal and one patient with multifocal hydatid cysts. The CD4 values were low (<200 cells/mm³) in all cases and we have not seen any response after continuously therapy with albendazole 800 mg/day, more than 6 months. In all cases surgery was required and was followed by complications such as hydatid peritonitis (two cases) and hydatid cholangitis (one case). Two relapses were reported in a patient with CD4<50 cell/mm³, one patient died due to complications.

Conclusions: In HIV-infected patients with cystic echinococcosis, with low CD4 count we have not seen any response after continuously therapy with albendazole 800 mg/day. We consider that CD4 count can be a predictive factor for response to treatment in all patients, influencing the choice of optimal therapeutic intervention method.

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A single-center experience on surgical treatment of hepatic echinococcosis

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Objective: To evaluate and discuss the various surgical methods for hepatic echinococcosis.

Methods: There were 402 patients with hepatic echinococcosis treated in West China Hospital of Sichuan University from 2009 to 2014, 271 of them underwent surgical treatment. The cystic echinococcosis was in 195 patients, including 80 cases performed classic endocystectomy or subtotal cystectomy, 109 performed total cystectomy or hepatectomy, and 6 cases performed palliative surgery. The alveolar echinococcosis was in 76 patients, including 7 cases performed palliative surgery, 54 cases performed hepatectomy, 12 cases performed liver autotransplantation and 3 cases performed liver autotransplantation.

Results: The draining time, the rate of postoperative complications, and the recurrence was (18.6±7.2) d, 21.2% (17/80) and 15% (12/80) respectively in the cases of cystic echinococcosis underwent classic endocystectomy or subtotal cystectomy, which were significantly higher than those cases of cystic echinococcosis underwent total cystectomy or hepatectomy (5.4±0.6) d, 7.3% (8/109), and 0.9% (1/109), respectively, P<0.05. The draining time and the recurrence was (5.9±0.7) d and 1.8% (1/54) respectively in the cases of alveolar echinococcosis underwent hepatectomy, which were significantly lower than those in the cases of alveolar echinococcosis took palliative surgery (9.7±1.4) d and 57.1% (4/7), respectively, P<0.01. The 12 patients underwent liver transplantation were complete rehabilitation, while the rest 3 were death.

Conclusions 1. Total cystectomy or hepatectomy should be the first choice for cystic echinococcosis; Palliative treatment could improve the symptoms of unresectable patients with cystic echinococcosis. 2. Hepatectomy should be the first choice for alveolar echinococcosis, palliative surgery could only be used to alleviate symptoms and physical signs and delay the progression of this disease. 3. Liver transplantation could be as a treatment option for late hepatic echinococcosis.
200 cases clinical introduction of hepatic cystic echinococcosis’s standard surgical treatment

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Objective: To explore the diagnosis and standard surgical treatment of hepatic cystic echinococcosis (HCE).

Methods: The clinical data of 200 patients who underwent the complete stripping surgery of echinococcosis in our department from January 2010 to January 2015. Results: 200 patients with hepatic cystic echinococcosis (HCE) were given the complete and standard stripping surgery of echinococcosis, these patients were followed-up from 6 months and 5 years; the effect was good. The surgical type solved these problems, including residual cavity infection, biliary fistula and residual cavity effusion.

Conclusion: Hepatic cystic echinococcosis’s standard surgical treatment is safe, reliable and can clearly, effectively reduce the postoperative recurrence rate and complications.

Keywords: hepatic cystic echinococcosis (HCE), standard surgical treatment, external capsule excision

Living donor liver transplantation for a complex hepatic cystic echinococcosis: a case report from China

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Objective: To have a summary of living donor liver transplant experience on treatment of complex hepatic Cystic Echinococcosis.

Methods: Retrospective analysis of a case of living related liver transplantation in the treatment of a Liver Cystic Hydatid disease combined blood stasis cirrhosis performed by first affiliated hospital of Xinjiang Medical University at 9th May, 2015.

Results: Living donor liver transplantation surgery is successful. The patient has implemented 4 times liver surgery for Cystic Hydatid disease, which lead to patient’s right liver lobe as deficient and left liver lobe significantly enlarged. Post-hepatic inferior vena cava and left liver vein are serious narrowed by press of current hydatid cysts. Post-hepatic inferior vena cava, portal vein and left liver vein became extremely tortuous caused by pushing of current hydatid cysts. Result of post-hepatic inferior vena cava and left liver vein radiography: Hepatic segment of inferior vena cava is seriously stenosis and left liver vein appeared as thin as hair, stent cannot pass and required surgery. The remaining liver nodulated and pathology is pathological false formed. Autologous liver transplantation will cause liver failure, so living donor liver transplantation was performed. Donor is the patient’s brother, operation taken right liver lobe (685g). After the operation, the patient recovered.

Conclusion: Cystic Hydatid disease of the liver as an infectious disease, hydatid cyst removal is the preferred method of treatment, when conventional surgery can not cure patient, liver transplantation as a treatment for this disease.

Analysis of 105 patients with hepatic cystic echinococcosis type 4-5

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Objective: This study is aiming to discuss the indication and timing of surgery in type 4-5 Cystic Echinococcosis (CE).

Methods: Clinical data of 84 patients with type IV and 21 patients with type V cystic echinococcosis were retrospectively analyzed. The surgery is determined based on the size, locations.

Results: Of the reported cases, 28 patients combined with cystic infection (26.63%), 8 patients with biliary rupture (7.61%), 18 patients with obvious bile fistula (17.14%), 23 patients with first hilum involvement (21.9%), 12 cases with Second hilum involvement (11.4%), 11 patients with third hilum involvement (10.47%). Simultaneous involvement of the first, second hepatic hilum was shown in 10.47% of reported patients; Co-involvement of first and third hepatic hilum was for 8.57% of reported patients.

Conclusions: The type 4-5 Cystic Echinococcosis were involved the large vessels hepatic portal area can affect hepatic blood reflux. The hepatic Cyst suppression of biliary duct and surrounding organs affect liver function. Infected hydatid cyst and ruptured cysts require emergent surgery.
Investigation of genetic diversity in *Echinococcus granulosus sensu stricto* using a new microsatellite polymorphism approach

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The *Echinococcus granulosus sensu lato* tapeworm is responsible for cystic echinococcosis (CE), a zoonotic disease with worldwide distribution causing economic losses in livestock. The genetic diversity in *E. granulosus s.s.* is mainly investigated by a classical approach of sequencing in order to observe punctual nucleotide mutations.

Using genome of *E. granulosus sensu stricto* recently available, a search of microsatellites was performed. After preliminary tests of amplification and migration by capillary electrophoresis, two out of the 15 microsatellites identified were selected (Sca6 and Sca11) according to their capacity of interpretation of the electrophoregram profiles obtained with *E. granulosus s.s.* samples. The in silico nucleotide composition of both microsatellites corresponds to those obtained after sequencing PCR products. The presence of one to four alleles for the Sca11 microsatellite has been confirmed and is due to the tandem repetition of this microsatellite according to the results of an in silico PCR performed on the *E. granulosus s.s.* genome. There is no amplification of *Taenia* spp. or *E. multilocularis* while profiles are obtained for all species among *E. granulosus s.l.* The discriminatory power was evaluated for the combination of the two microsatellite using 40 *E. granulosus s.s.* DNA from epidemiologically independent samples from sheep, cattle and humans from France and Tunisia. The Simpson index was evaluated to 0.990, with identification of 31 different profiles.

The potential of this tool was assessed by the investigation of the genetic diversity of 11 DNA samples isolated from CE cysts in liver and/or lungs from 2 different sheep breeding group (4 and 3 animals) originating from the South of France and slaughtered at the same period. Identical profiles were observed only for a maximum of two different animals. For the four animals infected in both liver and lungs, the same profile was reported in both organs only once.

This new microsatellites tool has the potential to be very helpful to describe genetic diversity in *E. granulosus sensu stricto*. Investigations at the individual level in animals but also in humans would bring new information concerning the origin and frequency of infection. Further studies need to be performed at different scales and endemic levels in order to assess the epidemiological interest of this tool.

Laparoscopic approach in liver hydatid cyst – possibilities and limits

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**Background:** Considering the risk factors for the post-operative complications for patients operated for hepatic hydatid cysts described in the literature, we compared the classical intervention and the laparoscopic one.

**Methods:** We performed a retrospective case-control controlled study on a total of 52 cases, 26 of which undertook laparoscopic surgery (case), 26 classical surgeries (control), operated between 2003 and 2009. The factors considered for the selection of the control group were: Gharbi type, preoperative biliary fistulae, gender, age interval (±10 years) and the intervention type. The factors that were compared between the two groups were: duration of the intervention, length of postoperative stay, intra- or post-surgery complications, drainage duration, recurrences, postoperative pain medication.

**Results:** The groups totaled 40 women and 12 men, ages between 14 and 70 years. Most of the cysts were Gharbi I (30.76%). The cyst sizes varied between 3 and 20 cm in the case group and 4 to 28 cm in the control one.

In the laparoscopic group duration of surgery (p=0.007), postoperative stay (p=0.011) and the period drains were removed (p=0.028) were significantly shorter. Major pain medication need was significantly higher in the open group (p=0.019, OR=0.8, CI 95%=0.7-0.9).

No intra operative complications were recorded. Major pain medication was necessary in 5 cases, all in the classical surgery group. Biliary fistulae appeared in 2 cases (7.69%) in the case group and 3 cases (11.53%) in the control group. There was one case of anaphylactic shock post operatory in the control group. There were no post operatory abscesses. There were no recurrences or reinterventions for the studied cases on a 3-year follow-up.

**Conclusion:** The laparoscopic intervention is recommended in selected cases, as it reduces the hospital stay, the impact on the abdominal wall and the postoperative pain, with no more complications than the classic one.

**Keywords:** hepatic hydatid cyst, surgery, laparoscopy, classic, comparison
Performance comparison of three rapid diagnostic tests for the serodiagnosis of hepatic cystic echinococcosis in humans

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The diagnosis of cystic echinococcosis (CE) is based on imaging, in particular ultrasound (US) for abdominal CE, complemented by serology when US features are unclear. In rural endemic areas, where expertise in US diagnosis of CE may be scant and conventional serology techniques are unavailable due to the lack of laboratory equipment, Rapid Diagnostic Tests (RDTs) are appealing. However, the performances of immunoassays are heterogeneous and influenced by many variables, and the interpretation of results may be difficult. We evaluated the performances of 3 commercial RDTs for the diagnosis of hepatic CE. Sera from 59 patients with single hepatic CE cysts (38 active, 21 inactive) and 25 patients with non-parasitic cysts were analysed by RDTs VIRapid HYDATIDOSIS (Vircell, Spain), Echinococcus DIGFA test (Unibiotest, China), ADAMU-CE (ICST, Japan), and by RIDASCREEN Echinococcus IgG ELISA (R-Biopharm, Germany). Sensitivity, specificity and ROC curves were compared with McNemar and t-test. For VIRapid and DIGFA, correlation between semi-quantitative results and ELISA OD values was evaluated by Spearman’s coefficient. Reproducibility was assessed on 16 randomly selected sera with Cohen’s Kappa coefficient. Se and Sp of VIRapid (74%, 96%) and ADAMU-CE (57%, 100%) did not differ from ELISA (69%, 96%), while DIGFA (72%, 72%) did (p=0.045). ADAMU-CE was significantly less Se in the diagnosis of active cysts (p=0.019), while DIGFA was significantly less Sp (p=0.014) compared to ELISA. All tests were poorly Se in diagnosing inactive cysts (33.3% ELISA and ADAMU-CE, 42.8% DIGFA, 47.6% VIRapid). ROC curves of VIRapid (AUC 0.851) and DIGFA (AUC 0.722) were significantly different (p=0.042). The reproducibility of all RDTs was good to very good. Band intensity of VIRapid and DIGFA correlated with ELISA OD values (r=0.76 and r=0.79 respectively, p<0.001). RDTs may be useful in resource-poor settings to complement US diagnosis of CE in doubtful cases. In this regard, VIRapid test appears to perform best among the examined kits, but all tests are poorly Se in presence of inactive cysts, which may pose considerable problems of differential diagnosis.

Cloning and characterization of potent Kunitz type protease inhibitors from Echinococcus granulosus

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Little is known about the molecular mechanisms whereby Echinococcus granulosus is able to survive in the hostile mammalian host environment, avoiding attack by host enzymes and evading immune responses, but protease inhibitors released by the parasite are likely implicated. We identified two nucleotide sequences corresponding to secreted single domain Kunitz-type protease inhibitors (EgKIs) in the E. granulosus genome and their cDNAs were cloned, bacterially expressed and purified. EgKI-1 is highly expressed in the oncosphere stage and is a potent chymotrypsin and neutrophil elastase inhibitor that binds calcium and reduced neutrophil infiltration in a local inflammation model. EgKI-2 is highly expressed in adult worms and is a potent inhibitor of trypsin. As powerful inhibitors of mammalian intestinal proteases, the EgKIs may play a pivotal protective role in preventing proteolytic enzyme attack thereby ensuring survival of E. granulosus within its mammalian hosts. EgKI-1 may also be involved in the oncosphere in host immune evasion by inhibiting neutrophil elastase and cathepsin G once this stage is exposed to the mammalian blood system. In light of their key roles in protecting E. granulosus from host enzymatic attack, the EgKI proteins represent potential intervention targets to control CE. This is important as new public health measures against CE are required, given the inefficiencies of available drugs and the current difficulties in its treatment and control. In addition, being a small sized highly potent serine protease inhibitor and an inhibitor of neutrophil chemotaxis, EgKI-1 may have clinical potential as a novel anti-inflammatory therapeutic.
The expression and significance of Foxp3 and NF-κB in hepatic tissues next to *Echinococcus multilocularis*

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**Objective:** To investigate the expression of Foxp3 and NF-κB in hepatic alveolar echinococcosis tissue and its relationship with clinico-pathological features of Tibetans with alveolar echinococcosis.

**Method:** Expressions of Foxp3 and NF-κB were determined in tissue samples from patients with hepatic alveolar echinococcosis by using tissue immunohistochemistry and the relationships between expression of Foxp3 and NF-κB with patients’ clinico-pathologic characteristics were assessed by chi-square test.

**Results:** A total of 20 Tibetan from Qinghai province, diagnosed as AE were enrolled in this study. Both Foxp3 and NF-κB showed a major nuclear staining and significant high positive rate in alveolar echinococcosis marginal band compared with corresponding normal liver (35% vs. 10% and 45% vs 10%, both P<0.05), however, there was no significant correlation between their expression and patients’ clinicopathologic characteristics (P>0.05).

**Conclusion:** Foxp3 and NF-κB may have considerable potential in identification of alveolar echinococcosis marginal band. Moreover, as their essential role in immune regulation, Foxp3 and NF-κB may play critical roles in pathophysiology of alveolar echinococcosis.

**Keywords:** Foxp3, NF-κB, alveolar echinococcosis, immune regulation

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The expression and significance of IL-17 and IL-27 in liver tissue next to *Echinococcus multilocularis*

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**Background:** Alveolar echinococcosis is a parasitic disease prevalent in pasturing area worldwide. In recent years, a rising number of studies have suggested that alveolar echinococcosis damage host’s liver through a malignant invasive way like a malignancy, and immune dysregulation may be closely related with its development. IL-17 and IL-27 are essential regulatory factors in human immune system. Therefore, we conducted this study to explore the expression and clinico-pathological significance of IL-17 and IL-27 in Tibetans suffering from alveolar echinococcosis.

**Method:** Expressions of IL-17 and IL-27 were determined in tissue samples from patients with hepatic alveolar echinococcosis by using tissue immunohistochemistry and the relationships between expression of IL-17 and IL-27 with patients’ clinico-pathologic characteristics were assessed by chi-square test.

**Results:** A total of 20 Tibetan from Qinghai province, diagnosed as AE were enrolled in this study. Both IL-17 and IL-27 showed a major cytoplasmic staining, meanwhile, they both showed a different extent staining in tissue space. Their expression (positive cell rate) are significant higher in alveolar echinococcosis marginal band than corresponding normal liver (80% vs 10% and 85% vs 20%, both P<0.01), however, there was no significant correlation between their expression and patients’ clinicopathologic characteristics (P>0.05).

**Conclusion:** IL-17 and IL-27 may have considerable potential as alveolar echinococcosis marginal band markers. Moreover, as their role in immune regulation, IL-17 and IL-27 may participate in pathophysiology of alveolar echinococcosis.

**Keywords:** IL-17, IL-27, Alveolar echinococcosis, immune regulation
The expression and significance of Smad2 and P38MAPK in hepatic tissues next to *Echinococcus multilocularis*

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**Objective:** To observe the expression level of Smad2 and P38MAPK protein in liver tissues of human with *Echinococcus multilocularis*, and to evaluate their interaction and the mechanism of signals in hepatic fibrosis.  

**Method:** 20 patients who were diagnosed with AE through surgery was recruited and their hepatic tissues were divided into para-lesion and normal liver tissues; Immunohistochemistry was applied to detect the expression of Smad2 and P38MAPK. Pathologic changes and the degree of hepatic fibrosis were evaluated using Hematoxylin and Eosin and Masson staining.

**Result:**  
1. The scores of hepatic fibrosis in para-lesions was higher than that in normal liver tissues (T=126, P<0.01);  
2. The expression level of Smad2 and P38MAPK in para-lesion were both higher than that in normal liver tissues (t=3.893, P<0.01; t= 4.675, P<0.01);  
3. The expression level of Smad2 and P38MAPK in normal liver tissues, para-lesion or the whole hepatic tissues were all positively correlated with the degree of fibrosis (P<0.05). The expression level of Smad2 and P38MAPK increased with the degree of fibrosis (P<0.05);  
4. The expression level of Smad2 was positively correlated with that of P38MAPK in para-lesion (P<0.01).

**Conclusion:**  
1. The degree of fibrosis in para-lesion with *Echinococcus multilocularis* is more serious than that in normal liver tissues;  
2. Smad2 and P38MAPK are both involved in the positive process of liver fibrosis;  
3. P38MAPK is involved in the positive regulation of Smad2.

**Keywords:** *Echinococcus multilocularis*, Smad2, P38MAPK, hepatic fibrosis

Preliminary findings of a widespread *Echinococcus* surveillance program in an endemic region in Turkey

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**Aim:** Among the clinical forms of the human disease, cystic echinococcosis (CE) is due to *Echinococcus granulosus*, a globally-distributed disease and a public health threat in Eurasia, including Turkey. In this study, prevalence of CE was investigated in regions where agricultural and animal breeding activities are frequent and previous information on the disease is limited.

**Material and Methods:** Four regions with different geographical and epidemiological properties, which include Cubuk district of Ankara province (Central Anatolia), Uzunkopru district of Edirne province (Eastern Thrace), Akcakale district of Sanliurfa province (Southeastern Anatolia) and Tatvan district of Bitlis province (Eastern Anatolia), were examined. The presence of CE was investigated via ultrasonography (US).

**Results:** Among the 3465 individuals examined, a total of 24 CE cases were identified. The frequency of cases by region was Akcakale (1.17%), Uzunkopru (0.82%), Cubuk (0.53%) and Tatvan (0.12%). The cases presented with US abnormalities involving liver (89.6%), kidney (2/29, 6.8%) and lung (1/29, 3.6%). Five individuals (17.2%) had two CE cysts. The size of the cysts was distributed within 1.23-13.94 cm.

**Conclusion:** CE activity has been demonstrated in all regions studied. Follow-up, laboratory testing and treatment of the current cases are underway.
Molecular identification of *Echinococcus granulosus* isolates from ruminants in Greece

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Data on the circulating *E. granulosus* strains in Greek livestock are scant. The objective of the present study was to present new data about the circulating *E. granulosus* strains in an extended area of the country and to provide a synoptic picture which could be used as a reference framework for future studies.

A total of 77 hydatid cysts collected from small ruminants and one cattle in 9 prefectures of the mainland of Greece and the islands of Euboea and Naxos (Aegean Sea) were analyzed through the polymerase chain reaction of a 450 base-pair segment of the Cytochrome C oxidase subunit 1 gene and sequenced directly in both directions. Seventy five of 77 samples were allocated to *Echinococcus granulosus sensu stricto* (cluster G1-G3). Thirteen haplotypes with 12 polymorphic sites were distinguished in the cluster. The majority of the generated sequences belonged to 8 common haplotypes identified in different parts of the world, while 7 samples were allocated to 5 unique haplotypes. In comparison to the predominant haplotype, the number of nucleotide changes in all the other haplotypes ranged from 1 to 5. Two samples from sheep in different regions (Arcadia, Ilia) in Peloponese were identified as *Echinococcus canadensis* G7 genotype. This is the first time that a large number of *Echinococcus* isolates from different parts of Greece are sequenced and the first report of the G7 genotype in sheep in the country.

Analysis of economic burden of patients with new model of personal case management of „doctors-nurses-social workers“

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Aim: Patients diagnosed and treated by new model of personal case management of „doctors-nurses-social workers“ understood the direct and indirect economic burden of the liver alveolar echinococcosis patients applying the new model, analyzed and explored its influencing factors.

Methods: Research object were surgical patients of liver alveolar echinococcosis in Xinjiang from a big hospital from June 2014 to June 2015, the information of patient's medical record and cost were input into the database, analyzed the data using the statistical description, variance, multiple stepwise regression method, then the indirect economic burden was estimated by the method of productivity weighted summation recommended by the combination of disability adjusted life years (DALY) and human capital method.

Results: the direct medical expenses of patients were 52538.86 yuan, the direct non-medical expenses per capita were 1352.60 yuan and the indirect economic burden per capita was 5364.98 yuan.

Conclusion: the economic burden of the liver alveolar echinococcosis patients under new model of personal case management was reduced slightly, but it was still heavier, the psychological burden of patients should reduce from the multi-angle care, pay more attention to the patients, improve the quality of patients’ life, shorten the average hospitalization days, strengthen the propaganda of hydatid disease knowledge, enhance awareness of prevention, increase the amount of subsidies, effectively reduce the patients’ economic burden.

Epidemic and control of hepatic echinococcosis in Kazak Autonomous Prefecture of Yili - a retrospective analysis of 2300 cases

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Objective: This study aims to analyse the nearly 10-year experience of diagnosis and treatment of hepatic echinococcosis (CE) in Kazak Autonomous Prefecture of Yili under the surveillance of „three-in-one“ hepatic echinococcosis prevention system with CDC and Health Bureau.

Methods: 1. The clinical data of 2300 case hepatic cystic echinococcosis patients from 2005 to 2014 were retrospectively analyzed; 2. The prevention, control and treatment of hepatic cystic echinococcosis is supervised by „three in one“ system by health administrative department, pointed hospital implementation. Local CDC is responsible for follow-up, Cooperation between the three parties, work together to prevention, control and treatment CE.

Results: 1. A total number of 1780 patients underwent surgery. Radical surgery was considered in 578 patients, with radical resection in 1020 patients and endectomy in 182 patients. No intra- or postoperative mortality were observed. Chemotherapy was considered in 380 patients with albendazole, meanwhile, 140 patients underwent “watch and wait” without special treatment. All patients followed-up carefully for 1-10 years. Among them, cystic echinococcosis reoccurred in 32 patients.

Conclusion: The „three-in-one“ in the prevention and treatment of hydatid disease in new system may improve the prevention, control and treatment of cystic echinococcosis and to foster the convergence of the three parties, to further standardize the treatment of CE prevention and control.

Keywords: Yili river valley, Echinococcosis prevention and control, trinity service system
Human cystic echinococcosis in Iran: the significance of camel-dog cycle

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More than ten species/strains/genotypes have been attributed to Echinococcus granulosus sensu lato of which sheep strain (i.e. E. granulosus sensu stricto, G1 genotype) is the dominant species in most parts of the globe. However the significance of camel strain (G6 genotype) in the epidemiology of human cystic echinococcosis (CE) is not fully understood. The camel, Camelus dromedarius, is a major intermediate host for E. granulosus in the Middle East and North Africa. The importance of camel-dog cycle of the parasite in terms of human infection and transmission patterns has been increasingly recognized during past two decades. In Iran a variety of animal intermediate hosts are involved in E. granulosus life cycle, including sheep, goat, cattle, camels and wild ungulates. In the present study the contribution of camel-dog cycle for perpetuating human CE in different regions of Iran is reviewed.

Human Formalin-fixed paraffin-embedded (FFPE) specimens from 182 individuals were collected from major hospitals in Tehran and Kerman provinces. Of 125 isolates sequenced on mitochondrial CO1 region, 41% were characterized as G6 genotype. In southeastern province of Kerman the number of humans infected by camel strain (46%) was more than that of sheep strain (42%). Characterization of two mitochondrial CO1 and ND1 genes in 42 freshly collected human isolates revealed G6 genotype as the causative agent of 57% of human CE cases. Canine echinococcosis was investigated on 307 stray dogs in Kerman. E. granulosus was found in 21 dogs (6.8%) by copro-PCR analysis. All dog specimens were shown to be G6 genotype. In contrast, all Echinococcus isolates from 71 dogs collected in western province of Lorestan were identified as E. granulosus sensu stricto. Thus camel strain seems to be rarely found in molecular investigations carried out in western and northern regions of Iran and the parasite is mainly perpetuated between two overlapping camel-dog and sheep-dog cycles especially in southern and eastern parts of the country.

Recovery indicator in human cystic echinococcosis/ hydatidosis patients after surgery by evaluation of their IgG and its subclasses IgG1 and IgG4

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Background: Echinococcosis is one of the most important helminthic zoonotic infection all over the world. The treatment of the disease is mainly surgery; however, no standard method for assessment of the patients after surgery is available. Immunoglobulin G subclass responses may be a better marker than total IgG, after surgery to follow-up of cystic echinococcosis (CE) patients.

Aim: To evaluate the value of IgG and its subclasses (IgG1 and IgG4) in cystic echinococcosis patients before and after surgery using ELISA.

Material & methods: A total of 30 CE patients were selected from Fars and Khouzestan provinces and evaluated for pre- and post-surgical treatment based on their clinical, radiological and laboratory tests for six months after surgery. The patient’s serum samples were collected before surgery, three and six months after surgery. A total of 12 sera from healthy controls were also included in the study. Total IgG and two subclasses including IgG1 and IgG4 were determined on all samples by enzyme-linked immunosorbent assays using native antigen B.

Results: Total IgG: The mean OD value before surgery, three month after surgery and six month after treatment was 3.03, 2.74 and 2.7 respectively with no significant decrease before and after surgery. IgG1: The mean OD value before surgery, three and six month after surgery was 2.2, 1.7 and 1.2 respectively. So, the mean of recorded OD started to decrease gradually within six months, but none of the patients changed to negative serology result after six months. Statistical analysis showed significant decrease level of IgG1 before and after surgery during these six months (P<0.05). IgG4: The mean OD value before surgery, three and six month after surgery was 3.46, 3.2 and 3.08 respectively. Its level was decreased, but not in a significant level.

Conclusion: The results show that evaluation of IgG1 level after surgery using ELISA with application of antigen B can serve as a helpful recovery marker in cystic echinococcosis patients.
**Echinococcus spp. and other taeniid cestodes in wildlife of southern Africa**

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Various species of *Echinococcus* and *Taenia* had been described in the past from wild mammals of sub-Saharan Africa. However, only few isolates have become available for molecular identification in recent years, which means that the involvement of wildlife in the lifecycles of the various cryptic species within *E. granulosus* sensu lato is still little known. Preliminary surveys were done in various conservation areas of Namibia in 2012-2014. Faecal samples were obtained from seven different wild carnivore species and carcasses of eleven different culled herbivore species were examined for larval taeniids. A second survey was undertaken in the Kafue National Park, Zambia, in 2013, where faecal samples of five different wild predator species were opportunistically collected. In Namibian wildlife four *Echinococcus* species could be found, namely *E. equinus*, *E. felidis*, *E. canadensis* and *E. granulosus* and additionally *E. ortleppi* in cattle. In comparison to this high variety in Namibia, *E. felidis* was the only *Echinococcus* species in the Zambian sample collection. Further, a variety of sequences was obtained from eggs and cysticerci from lions, cheetahs, caracals, spotted hyenas and oryx, which most closely clustered with species of *Taenia* and *Hydatigera*. Only three of them, of lion and hyena origin, could be allocated to *H. taeniaeformis* and *T. regis* in lion and *T. crocutae* in hyena, respectively. *T. regis* cysticerci were also found in oryx. The remaining sequences were tentatively assigned to 12 distinct *Taenia* taxa, but could not be further identified due to nonavailability of intact adult worms and the lack of Genbank data for most African *Taenia* species. The diversity of other taeniids in wild mammals was found to be far higher than expected. This study confirms the presence of all African *Echinococcus* species in Namibian animals and identified the first *Echinococcus* species in Zambian wildlife.

**Repeated infection of dogs with protoscoleces of Echinococcus granulosus induced high resistance against adult worm infection**

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**Background:** Dogs play a pivotal role in the transmission of hydatid disease which is a near cosmopolitan zoonosis and significantly impacts on population health and animal production in the endemic areas. Dog vaccination against the tapeworm infection will benefit the control of the disease.

**Methodology/Principal Findings:** To determine whether dogs can obtain protection against *E. granulosus* through repeatable infection, we performed 5 runs of infection/purge to 17 beagles. For infection, each dog was orally given 220,000 protoscoleces and after 35 days of infection, the dogs were purged with arecoline hydrobromide and all the eliminated worms released in feces were counted. Peripheral blood mononuclear cells (PBMC) were collected for detecting cytokine gene expression level by real time PCR and serum was collected for testing specific antibodies by ELISA. The first infection induced 90% protection in term of worm reduction compared to the worm numbers of the first infection and the last infection had only 3% of worm number of the first infection.

**Conclusions/Significance:** Dogs can be induced high level of protection against infection by repeated infection, which is important for dog vaccine development against *E. granulosus*.
A European survey of the treatment of biliary complications by per-endoscopic procedures in patients with alveolar echinococcosis

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Aim: Biliary complications occurrence indicates a turning point in the course of Alveolar Echinococcosis (AE), a severe cancer-like parasitic disease. This retrospective European study aimed at providing data on the current usage of per-endoscopic procedures (PEPs) to treat biliary complications of AE, its technical aspects, as well as on the patient’s outcome after PEP.

Patients and methods: Data regarding patient’s characteristics; AE history, staging according to PNM international classification, previous treatments, symptoms preceding PEP, biological and microbiological parameters, per-endoscopic techniques, associated treatments, and follow-up were recorded using an online questionnaire. Last follow-up data were recorded on January 31st, 2015.

Results: From 19 centers, 45 patient files, from 1997 to 2014, could be analyzed, including 167 PEPs. The first PEP was concomitant to AE diagnosis in 62% and performed within the first 3 years after diagnosis of AE in 76% of patients; 38% had previous surgical interventions for AE. Biliary stones were found in 25% and purulent bile in 19% of procedures. Resolution of the initial symptom(s) was obtained in 95% and long-term biliary duct patency in 78% of cases. Complications of PEPs were similar to those observed in other indications, except for cholangitis which was more frequent (11%), especially in the absence of lavage and/or antibiotics administration. Stenting was performed in 31 patients, a combination of stent placement and stricture dilations in 11, and sphincterotomy alone in 3. Repeated plastic stent placement was the most common procedure: 155 plastic stents were inserted during 90 PEPs; median time between 2 stent placements was significantly longer when 3 stents or more were placed.

Conclusions: Most referral centers in Europe use PEP routinely to treat AE biliary complications. Extensive lavages of the bile ducts and antibiotics should reduce cholangitis. Insertion of multiple plastic stents (at least three) delays stent occlusion and leads to effective and prolonged bile duct patency.

The role of the rodent in the Echinococcus multilocularis lifecycle in Sweden

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Although Echinococcus multilocularis was first identified in a red fox (Vulpes vulpes) in Sweden (2011), the rodent intermediate host(s) remained unknown. The aim of our study is to investigate potential rodent intermediate hosts to determine their role in the E. multilocularis lifecycle in Sweden. Understanding the rodent host(s) and their ecology is important to understanding the ability of the parasite to exist in the environment. In particular, the absence of one of the most common European intermediate hosts, Microtus arvalis, could limit the presence of E. multilocularis in Sweden.

Rodents were trapped in four study areas in southern Sweden during spring and fall 2013-2014 using snap and topcat traps. After storage at -20˚C, rodents are dissected with special focus on the liver. All suspect parasitic cysts in the liver are identified with a multiplex PCR specific for E. multilocularis and taeniid species and all positive PCR results are sequenced.

A total of 1628 rodents of the species Arvicol a amphibius (n=396), Microtus agrestis (n=166), Myodes glareolus (n=644), and Apodemus spp. (n=422) were collected from the field. Dissections are ongoing. Preliminary results have identified E. multilocularis cysts (confirmed by PCR, sequencing and histology) in three A. amphibius and one M. agrestis to date. Protoscoleces, indicating mature cysts infective to the definitive host, were identified in the M. agrestis and one of the three A. amphibius. All four positive animals were found in one study area.

This study is the first to describe E. multilocularis in rodent intermediate hosts in Sweden. Our results show that M. agrestis and A. amphibius are suitable intermediate hosts for the tapeworm in Sweden. The absence of positive findings in M. glareolus and Apodemus spp. examined thus far indicate that these species are of little or no importance for the tapeworm’s lifecycle in Sweden. Furthermore, E. multilocularis is unevenly distributed in the environment. By better understanding the preferred rodent intermediate host, we may provide a basis for modelling parasite presence in the environment and, ultimately, predicting the risk for human exposure in the future.
Review of cystic echinococcosis (CE or hydatidosis) control programs and eradication campaign all over the globe in the last 40 years – personal involvement

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Hydatidosis is an important parasitic disease caused by a specific tapeworm, Echinococcus granulosus, which is one of the most important cestode infection causing morbidity and mortality in humans and livestock and significant economic losses in livestock. The parasite maintained primarily in humans and domestic animal cycle with dogs being final host and Ungulate animals, i.e. sheep, goats, cattle, camels etc. are the main intermediate hosts. In the intermediate host (humans and animals) the disease results in the development of large parasitic cysts in the viscera and on the organs. E. granulosus has broad geographic distribution and recent review indicates the spread of infection into areas previously free of it. Extremely high prevalence at herding area of Asia, Africa, South and Central America (Uruguay), Europe (Cyprus, Greece) and the Middle East countries. The poor sanitation and hygiene plus unrestricted disposal of animals’ disposal viscera post slaughter may be responsible for the high prevalence of human CE.

We review hereby our cooperative project with different control programs and projects; we also describe the different systems used in each country, accordingly to the habit and cultural behavior of specific population. We also take part in the process of the use and production of the recombinant vaccine EG 95 (David Heath, N.Z).

We also have done serological and molecular tests with dogs’ feces; Elisa test with P. Craig from Salford University, U.K and PCR test with Hamburger Hebrew University, Israel.

In conclusion, we have visited different control systems in many countries (with help of NIH grant (U.S) and P. Schanz from C.D.C.). Some have implemented successful control programs against cystic echinococcosis. We hope that these effort and knowledge gained during the last decades will help incentive for continued efforts to develop and implement preventive control measures against this preventable disease.

The susceptibility of the red foxes to infection with Echinococcus species in Romania

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Tapeworms of the genus Echinococcus are important parasites of mammals, causing life-threatening diseases. Both diseases, cystic and alveolar echinococcosis, are listed among the most severe parasitic diseases in humans, representing 2 of the 17 neglected diseases prioritized by the World Health Organization.

However, little is known about the role of red foxes in transmission of both parasite species. There is, to date, no evidence of positive cases of E. granulosus in red foxes from Romania but in most of Eastern Europe the parasite is considered to be endemic especially in dogs. Regarding E. multilocularis in red foxes, the parasite was described in Romania first in 2011.

Consequently, the copro-prevalence of these parasites through classical and molecular techniques in foxes from 9 counties from Romania was evaluated in this study.

Through coproparasitological examination (flotation and sedimentation), 18.9% of cases (41/217) were found to be positive for Taeniidae infection. The highest prevalence was observed in Harghita county (42.9%).

For each copro-DNA sample extracted from red fox feces, a 373-bp segment of rrsS mDNA was used to screen for infection with Taeniidae family. Nested polymerase chain reaction analysis was used to target a 255-bp segment of the ribosomal 12S gene to distinguish E. granulosus genotype G1 and a 250 bp fragment for E. multilocularis. Among 217 fecal samples, 127 (giving a copro-prevalence of 58.5%) showed the presence of Teniidae spp. In the second reaction of nPCR, 39 (18%) of samples were found to contain E. granulosus genotype G1. No fox feces were found to be infected with E. multilocularis.

Red foxes should be regarded as potential definitive hosts of the strains of E. granulosus and a potential risk to human health.
Microdiversity of *Echinococcus granulosus sensu stricto* in Australia and Chile

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**Aim:** To study the microdiversity of *E. granulosus* in Australia and Chile based on the sequences of the full length *cox1* gene.

**Material and Methods:** In the case of Australia, a total of 34 samples consisting in individual hydatid cysts from domestic animals (sheep and cattle), wildlife (wallaby, kangaroo) and adult specimens (dingoes) were obtained from different regions in Victoria, New South Wales, Australian Capital Territory, Tasmania and Queensland. In the case of Chile, a total of 90 hydatid cysts from domestic animals (sheep, cattle, goat and pig) and also 18 human samples were included in the study. The *cox1* gene was amplified in two steps with specific primers. The full length sequences of the *cox1* gene were used for the study of the microdiversity of *E. granulosus* and haplotype network construction.

**Results:** Microdiversity of *E. granulosus s.s.* is higher than expected in Australia, the study has found at least five haplotypes not previously described elsewhere. In the case of Chile, a similar situation occurs, microdiversity is higher than data previously reported in the continent and new haplotypes were discovered.

**Conclusion:** Microdiversity of *E. granulosus* in Australia and Chile is higher than expected. This study confirms the need to continue studying the microdiversity of this parasite in different geographic areas. It also shows how important is to compare the full length of the *cox1* gene instead of partial sequences of this gene. Implications of this variability in both countries will be discussed.

Surgical approach for pulmonary hydatid cysts – 30 years of experience

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**Aim:** Pulmonary hydatid cyst is a common pathology in children coming from the rural areas. In this paper we evaluate the results of the surgical treatment over the past 30 years.

**Material and methods:** We present a retrospective study and the management results of children with pulmonary hydatid cysts treated between 1984 and 2014 at our institution. Of 462 children with pulmonary hydatid cysts, 85% had unilaterally lesions, while in 15% of cases both lungs were affected; the right lung was involved in 54% of the cases. In 74% of the cases there was a single cyst and in 26% there were multiple hydatid cysts. In 59 cases there were associated extrapulmonary hydatid cysts. The treatment consisted of cystotomy with wedge resection, membrane removal, drainage of the remaining cavity and of the pleural space in 87.5% and ideal cystectomy in 11.5%. Thoracic-phrenic-laparotomy was performed for associated liver and right pulmonary cysts in 1% of the cases.

**Results:** We had postoperative complications such as wound infection, prolonged bleeding and one death in a 2-year-old boy with multiple pulmonary bilateral cysts. The drainage of the remaining cavity was prolonged in 8 cases. There was no recurrence of the hydatid disease.

**Conclusion:** Pulmonary hydatid cyst is the most frequent surgical pulmonary disease in children in our country. All hydatid cysts were incidentally discovered. Our approach was lateral thoracotomy without rib resection. In bilateral lung localizations, the second intervention was performed 3 to 6 months after the first. The treatment of the pulmonary cysts had priority on the extrapulmonary localizations.
Using the minimally invasive techniques for the treatment of hydatic hepatic disease: implementation of HERACLES EU Project

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Background: PAIR (Puncture, Aspiration, Injection, Re-Aspiration) is considered in this moment the standard treatment method for hydatic hepatic cysts larger than 5 cm, types CE I, CE II according to WHO-Informal Working Group on CE (WHO-IWGGE) classification. It was proposed in 1986 by a Tunisian team leaded by Ben-Amor and subsequently implemented in the 90’s by Filice and Brunetti in Italy. Placing a catheter at the end of the procedure (PAIR-D) is recommended for larger cysts. Akhan, in Turkey, has modified this technique increasing the catheter’s diameter and setting it under fluoroscopic control (MoCAT), using it successfully for type CE 3a and CE3b cysts.

Material and methods: As a part of the European Project FP7 HERACLES, in the General Surgery Clinic of the Colentina Clinical Hospital, a major step has been done to implement this kind of minimal invasive techniques in order to treat the intraabdominal hydatic disease.

During 03.2014-07.2015 period, 14 Mo-CAT and 17 PAIR procedures have been performed at 28 patients (2 patients had two hydatic hepatic cysts and two procedures were performed; other 2 patients needed the second same type procedure during the surveillance).

Results: The course of the disease was favorable and the patients remained under surveillance the next 2 years post-intervention, according to HERACLES protocol. There were no major adverse effects. In a single case that underwent MoCAT procedure an abscess of the residual cavity has developed, which has been drained percutaneously. Using MoCAT for other 2 patients, who were diagnosed with relapse of the hydatic cyst, we were able to prove and also to treat abscesses of the residual cavities. The same type of drainage was used to treat a residual cavity after PAIR, which didn’t shrink after 1 year, due to a biliary fistula. The results were also good.

Discussion: We believe the mini invasive techniques as PAIR and MoCAT are indeed methods of election to treat the hydatic hepatic cysts. Their results are good and invite us to use these techniques as the first choice; the role of the open surgery remains for those cases with severe complications. Even the difficulties during the evolution of the remaining cavities (abscesses, lack of remission) could be solved with the mini invasive techniques.

Keywords: hydatic cyst, mini invasive techniques, PAIR, Mo-CAT, residual cavity

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Neglected diseases returning into the attention of national public health authorities through international projects (case study HERACLES Project)

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Aim: Even if parasitic diseases are neglected because of poor resource allocation, lack of surveillance, difficulties in prevention and control, their morbidities start to raise concern among public health authorities. As partner in HERACLES collaborative project, we want to bring out the current situation on cystic echinococcosis (CE) in Romania and contribute to fight this disease.

Material and Methods: National statistics, published articles and clinical records of Colentina Clinical Hospital on CE were used to select rural areas to screen the population according to HERACLES protocols. One of the main tasks of HERACLES collaborative project is training local rural population during the surveys. Therefore, we organized a series of field activities directed to the local communities involved in the survey to disseminate knowledge regarding the prevention and control of CE.

Results: Under Work Package 1 of HERACLES project, ultrasound surveys were conducted in Giurgiu and Brăila counties. Brochures reporting basic information about CE (parasitic life cycle, risk factors, prevention and control) were distributed in order to raise public knowledge on parasite epidemiology. These preventive medicine activities focusing on how to acquire and prevent CE were carried out in 14 villages. Participants to the ultrasound screening signed the informed consent and responded to a questionnaire focusing on possible pathways to acquire this disease. Consequently, 2,902 patients were screened in 14 days during June-July and September-October 2014. The team was provided with a “mobile lab” that allowed blood samples collection and storage according to HERACLES protocols.

Conclusions: Since CE is usually asymptomatic for years, an active search by ultrasound screening is needed, in order to quantify the real burden of this disease. Considering the human and technical resources needed to cure and follow-up CE patients, screening remains a useful and affordable approach for active cases detection and for treatment allocation. The preventive medicine activities such as training and dissemination are very important to raise awareness on this neglected parasitic disease. Because of the lack of a national program targeting CE, the interventions provided with the aid of European Programs, funded through HERACLES project, is a valuable add on to the health care of the countries involved in the research. The results obtained will support the health authorities in order to establish sustainable measures for CE prevention and control in our country.

Keywords: neglected diseases, intervention, European projects, HERACLES project

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Monitoring HERACLES prospective patients and prepare their registration in the European Register for Cystic Echinococcosis (ERCE)

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Aim: Human cystic echinococcosis (CE) remains a neglected disease, despite the major contributions of recent medical advances to this subject. Its prevalence continues to increase alarmingly, therefore patient management needs to be improved, for a better surveillance program. We carried out a prospective cohort study, in which data collection started from 2014, as part of HERACLES collaborative project.

Materials and methods: The enrolled population in this study included 135 Romanian patients, with CE currently present in the medical records of Colentina Clinical Hospital, Bucharest. These patients are from both urban and rural areas, in almost equal proportions and have a variable level of education.

Results: Upon a preliminary assessment, patients aged between 40 and 60 years old were the most affected by CE (35%), with a higher female prevalence rate (55% women, 45% men). The most frequent residential areas were Bucharest and Călăraşi county. Serum samples tested positive using ELISA in almost two thirds of the patients.

Hepatic cysts (HC) were mainly encountered in 90% of the patients, but there were also cases with other localization, at lower rates (8 pulmonary, 7 in kidney, 5 in peritoneal, 6 splenic, 1 mediastinal, 5 in bones, 1 in muscles). Moreover, one quarter of the patients had multiple HC. Regarding cyst stages (according to WHO-Informal Working Group on CE, WHO-IWGE), the most often encountered during hepatic ultrasound were CE1 (30%), followed by CE3 and CE4, in similar proportions (roughly 25% each). The majority of the HC were discovered in the right hepatic lobe, particularly in segments 7-8. Twenty percent of the patients are currently under a Watch and Wait approach according to WHO-IWGE, 27% received only antiparasitic drug therapy with albendazole, 40% went under surgical intervention and 13% underwent percutaneous treatment. Among our patients, we found several cases of disease recurrence (12%) following surgery or PAIR.

Conclusions: Hydatid disease represents a major public health problem in Romania. Hydatidosis treatment is individualized. The follow-up of patients for several years is important, depending on the number, localization and dimension of the hydatid cysts. Each cyst could have an independent evolution. Interdisciplinary collaboration, in specialized centers is extremely important.

Keywords: cystic echinococcosis, monitoring, European Register

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Evolution of minimally invasive surgery in the management of hepatic hydatid cyst

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Despite its good results reported since 1991, the minimally invasive surgery (MIS) of hepatic hydatid cyst is still of limited use.

MIS for HHC can be made by totally laparoscopic surgery, laparoscopic assisted surgery or Endoscopic Laparoscopic Surgery (ELS).

Aim: To analyze the literature about MIS and the surgical incidences in endemic and hyper-endemic countries and report results of our 307 cases of HHC managed by laparoscopic assisted surgery.

Results: MIS is adopted in 29 countries. Our series included 307 cases of HHC, with 226 females, 81 males, mean age: 38 (16-70 years). Conversion: 66 cases (21.49%). Morbidity: 11 biliary fistulas, 1 bilious intraoperative épanchement, 1 biliary peritonitis, 12 infections of the residual cavity, 3 wound infections, 4 cases of unexplained fever. Postoperative mortality was nil. One death appeared, 4 months after surgery due to subphrenic abscess. Average hospital stay was 5 days. Two local recurrences appeared, after a mean follow-up of 59 months. No spillage was registered in this series and no anaphylactic shock.

Conclusion: Laparoscopic assisted surgery with no risk of spillage allows enlarging the indications. Totally laparoscopic surgery could be simplified and made more effective if a scleroidal agent could be found that melts the entire endocyst without causing harm to the biliary epithelium.
Surgical treatment of the abdominal hydatid cyst – 5-year experience from Colentina Surgical Clinic

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Introduction: In Colentina Surgical Clinic, hydatid disease beneﬁts both of diagnosis and treatment that allows individualized therapeutic approach. This multidisciplinary approach depends on the stage of the disease and it is represented by drug therapy, minimally invasive therapy, surgery or combinations of these therapeutic methods. For inactive hydatid cysts, the "watch and wait" approach may be used. The Surgery Clinic from Colentina Hospital, in collaboration with the Parasitology Clinic, has one of the largest casuistry in the country regarding the treatment of abdominal hydatid cyst.

Material and method: In the Surgery Clinic of Colentina Hospital, between 2010 and 2014, 146 patients with abdominal hydatid cyst were hospitalized and underwent interventional treatment. Among these, 124 patients had strictly intrahepatic localization, 12 had mixed intra- and extra-hepatic localization and 10 were diagnosed with extrahepatic localization. In patients with hepatic hydatid cysts, there were practiced both radical and partial resection, external drainage of the remaining cavity or pericystogastrointestinal anastomosis and minimally-invasive interventions (PAIR, MoCAT).

In cases with extrahepatic location, splenectomy, omentectomy, partial or total cystectomy were performed, depending on the location of the cyst. From a total of 146 interventions, 119 were carried out in the open technique, 8 laparoscopic, 5 cases required conversion to laparotomy after laparoscopy and in 14 cases minimal-invasive techniques were performed. Thirty-eight patients with external drainage of the remaining cavity beneﬁted from postoperative endoscopic sphincterotomy. The postoperative evolution was favorable, except for two cases, which required reintervention to solve abscesses developed on the remaining cyst cavities.

Results: Most interventions for liver location of the hydatid cyst were ﬁnalized by creating an external drainage of the remaining cavity. For extrahepatic located cysts, the intervention technique was inﬂuenced by the extension of the disease.

Conclusions: In cases with remaining cyst cavity, the internal drainage provides better postoperative evolution. Postoperative endoscopic sphincterotomy improves the healing process of the remaining cavity. Minimally invasive methods, with strict indications, are an alternative to surgery.

Keywords: hydatid disease, surgical treatment

A microarray analysis of *Echinococcus granulosus* transcription showing the early adult worm and cyst development from protoscolex stage

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A distinguishing feature of the biology of *Echinococcus granulosus* is that the larval protoscolex (PSC) can differentiate either (a) into an egg-producing adult worm (in the definitive dog host) or (b) into a new asexually reproducing hydatid cyst (in mammalian intermediate hosts or humans). To determine the transcription profiles in the early bi-directional development, we employed microarrays analysis to quantify expression levels of 12999 genes. We identified 4069 genes (31.8%) up-regulated in either adult or cyst development with fold change more than 2 compared to control PSC. Among the genes, 249 genes were from pre-adult in 3 hours; 650 genes in 12 hours and 1069 genes in 24 hours after PSC cultured with bile salt. Ninety nine genes were specifically expressed in adult worm with fold change more than 50 compared to PSC and 49 with predicated functions including 10% of the genes which are phosphorylases. Two genes with highest fold change to PSC are egM123 and egM9, being 2,680 and 2,522 times higher, respectively, in fold change. In hydatid cyst development, 104 genes were up regulated with more than 50 fold-changes compared to PSC, among them, 11 genes are encoding ribosomal proteins and 6 genes are antigen B.

The efficacy of radiotherapy to cystic echinococcosis in sheep

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**Background and Aims:** Radiation therapy is one of the three methods for treating cancers and more than 50% of cured cancer cases are treated by this therapy. In the study we used radiation therapy to treat sheep hydatid disease caused by *Echinococcus granulosus*.

**Methodology/Principal Findings:** We used ultrasonography to screen sheep flocks in an echinococcosis high endemic area in Xinjiang, China and 20 sheep were found infected with *E. granulosus* cysts in liver and/or lungs. The size and position of the cysts were confirmed by CT scanning. The sheep were randomly assigned into three experimental groups received high (60Gy), medium (45Gy), low dose (30Gy), respectively and one control group received no X-ray irradiation. Each of the target cysts had three radiation treatments in one week. After 3 months of the radiotherapy, CT scanning was used to measure the cyst condition including the size and image density. All the sheep were sacrificed and the livers and lungs were removed. Tissue samples including parasite cysts and host tissues were collected for histological and gene expression tests.

CT scan image showed that in the high dose group the irradiated cysts in 4 sheep were calcified and the cyst in one sheep was not changed. In the medium dose group, the irradiated cysts in 4 sheep remained the same and the cyst in one sheep was calcified. The results of the low dose group were similar to the medium group. HE staining showed that the irradiation damaged cyst structure and nucleus in the germinal layers were dissolved or disappear. Quantitative PCR results showed that EgTPx expression was decreased in a dose depended pattern (P<0.05); EgEPC1 was expressed higher in the low-dose irradiated cysts than that in the high-dose group (P<0.05); the expression of EgHSP70 was no significantly changed between groups (P>0.05). Between control and experimental groups, sheep physiological parameters such as general living conditions, body weight, liver function, electrolytes had no significantly change (P>0.05).

**Conclusions:** Radiotherapy may be a method for treating liver and lung cystic echinococcosis.
Aim: Comparative evaluation of the indirect hemagglutination assay (IHA) and two qualitative enzyme-linked immunosorbent assay (ELISA) for cystic echinococcosis (CE).

Material and Methods: The study included testing initial sera from 92 patients from Serbia with cystic lesions of the liver (on ultrasound-US) at the Parasitological Laboratory, Clinical Center of Serbia, from August 2013 to February 2015. Cellognost-Echinococcosis IHA (Siemens, Germany) was used to screen all patients. Results ≥1:64 were considered positive when hemagglutinins against type O erythrocytes were absent. Patients were separated in two groups that were in different time periods tested by different ELISA. In group 1 (n=51) Vircell® ELISA IgG (Spain) was used. Result was considered to be positive if the index of positivity was >1.1. Group 2 consisted of 41 patients tested by Ridascreen® ELISA IgG (R-Biopharm, Germany). Specimens with index of >1.1 were considered positive. Kappa (k) statistic, by Landis and Koch, was used to estimate of the tests’ results agreement.

Results: The study included 33 men and 59 women, mean age 54.2±17.5, range 11–78 years. Agreement between IHA and Vircell® ELISA was 84.3% (43/51), (k=0.73, 95% CI 0.57-0.89) and IHA and Ridascreen® ELISA 90.2% (37/41), (k=0.80, 95% CI 0.62-0.98). For both tests kappa estimates were reflective of ‘substantial’ (0.61-0.8). The concordance rate with both IHA/ELISA-positive (n=42), intermediate (n=5) or negative (33) results was 87% (80/92 sera). Twelve discordant results included: 3 ELISA positive/IHA negative; 1 ELISA positive/IHA +/- (1:32); 2 ELISA negative/IHA positive; 4 ELISA negative/IHA +/-; 1 ELISA +/- /IHA positive; 1 ELISA +/- /IHA negative. Combining two serological tests (42 positive with both IHA/ELISA and two positive by one and intermediate by other test) diagnosis of echinococcosis was confirmed in 44 patients (13 men and 31 women, mean age 52.1±17.5, range 11–78 years) and treatment with albendazole was started. They serologically monitored at 6 months. Therapeutic approach in patients with discordant/negative results depended of characteristic cysts by US and other imaging techniques.

Conclusion: The present study demonstrated a substantial agreement of results between IHA and two different ELISA. Positive results with both IHA/ELISA can confirm the diagnosis of CE. Objective disadvantages of the applied serological methods and discordant results required the application of more sensitive methods like immunoblot.

Molecular characterization of Echinococcus granulosus isolates obtained from different sources

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Cystic echinococcosis (CE) is a parasitic disease caused by the larval stage of Echinococcus granulosus. CE is more prevalent in developing countries where pasture animal husbandry is common. CE is also an important source of outcome and a serious health problem in our country. Humans are accidental intermediate hosts and are not able to transmit the disease. Up to now, molecular studies have showed that there are 10 distinct genetic types of E. granulosus. The aim of the study was to characterize isolates of the E. granulosus cysts in human and different animals in Turkey. This study was supported by Erciyes University Scientific Research Projects Unit, Turkey (Project No. TYL-2013-4329).

A total of 41 E. granulosus isolates were collected from 25 humans, 8 cattle, 6 sheep and 2 goats. Genomic DNA was extracted from protoscoleces using by genomic DNA purification kit according to the manufacturer’s instructions.

Genomic DNA was amplified using two mitochondrial targets (Cox1 Shorter fragment (S) and Cox1 Longer fragment (L)).

After DNA sequencing of Cox1 nucleotide, sequence analysis was undertaken by BLAST algorithms. Multiple sequence alignments were made with ClustalW method and MEGA version 6.0 software. Results were compared with the sequences retrieved from GenBank.

As a result, BLAST and Phylogenetic analyses of DNA isolation announced from Turkey were found to be similar to each other. All isolated our samples were determined as parasite’s domestic sheep strain (G1). However, this work also needs to be supported with the large number of DNA isolates obtained from different regions and animals.
In vitro efficacies of new thiourea derivate against Echinococcus granulosus cysts

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Introduction: Echinococcus granulosus is a cestode parasite. The metacestode stage causes cystic echinococcosis (CE) mainly in the human liver and lung. Current chemotherapy against CE is based on mebendazole and albendazole. However, benzimidazoles result in a low cure rate or are ineffective in many patients; therefore, novel compounds for the treatment of this disease have been studied.

As new drug targets the antimicrobial and antiparasitic effects of some thiourea derivates were reported previously. We aimed to show the antiparasitic effects of new thiourea derivates and create basic information for future studies in this study.

Material and methods: The new thiourea derivates were synthesed in Pharmaceutical Chemistry Department laboratories of Faculty of Pharmacology belonging to “Carol Davila” Medicine and Pharmacology University. The effects of new thiourea derivates against Echinococcus granulosus protoscolex cultures were calculated using 7 different concentrations (200µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL) in in-vitro conditions.

Results: The death of whole parasites was achieved with 72 derivates (70,52%) in one of the 7 concentrations in Echinococcus granulosus trials. Additionally, the number of the alive parasites was decreased with 9 (8,82%) derivates and the number of the alive parasites was not changed with 21 (20,58%) derivates.

Conclusion: As a future aim, there is a need to study the toxicity assays of the effective derivates and go on in-vivo derivate assays using cell cultures and/or animal models of this parasite.

Keywords: Thioureides of the 2-(4-methylphenoxy)methyl]-benzoic acid; thioureides of the 2-(4-ethylphenoxy)methyl]-benzoic acid; Echinococcus granulosus

In vitro and in vivo activities of insulin receptor signaling pathway inhibitor PQ401 against protoscoleces and vesicles of E. granulosus

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Background: During the process of the lava of E. granulosus parasite in the intermediate host, the complicated signaling pathway of E. granulosus helps the helminthes to evade from the immune system of the host. The insulin receptor signaling pathway, which could regulate the development, growth, glucose metabolism and motility of various parasites, is a crucial pathway in the parasite and becomes anti-helminthes drug targets.

Material and Methods: In order to study the relationship of the insulin receptor signaling pathway and the viability of the larva of E. granulosus, insulin receptor signaling pathway inhibitor PQ401 was used to treat the protoscoleces, vesicles and cystic echinococcosis mice and the impacts of PQ401 on the protoscoleces and host organs were evaluated by eosin staining, transmission electron microscopy (TEM), MTT, blood glucose, body weight, hepatic and renal function tests and vital organs (liver, kidney, lung and brain) pathological structure observation.

Results: The results of experiment in vitro showed that, among the 3 inhibitors (OSI-906, BMS-536924 and PQ401) of insulin receptor signaling pathway, PQ401 has the strongest effect on the viability of protoscoleces. The inhibition effect of PQ401 on the vesicle viability is superior to the protoscoleces, because when treated with 12.5 µM PQ401 for 2 days, vesicles all died, while protoscoleces were treated with 25 µM PQ401 for 4 days and 6.1% protoscoleces were still alive. The TEM results show that PQ401 mainly damaged cell membrane and nucleus of protoscoleces and vesicles. The MTT results showed that PQ401 has little effect on the cell proliferation of human liver cell 7702 and human embryonic kidney cell HEK293T. The results of experiment in vivo showed that the weight and number of vesicles in the PQ401 high dose treatment group (100 mg/kg) were significantly lower than control group (P< 0.05). TEM results showed that cell membrane was rupture, nucleolus disappeared and vesicle epithelial was necrosis, in the PQ401 high dose treatment group. However, PQ401 has little impact on the blood glucose, body weight, liver, kidney function and pathological structure (such as liver, kidney, brain and lung organ) of mice.

Conclusions: The intervention of the insulin receptor signaling pathway could inhibit the activity of the larva. This work laid a foundation for developing the new anti-echinococcosis drug.

Key words: Echinococcus granulosus; Insulin receptor signaling pathway; Inhibitor; Viability
Chemotherapy of human liver cystic echinococcosis in Bulgaria – a thirty-five-years-long experience

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Echinococcosis is a chronic disease in humans. Surgery is the most frequently used method of treatment of patients with hydatid disease. Thirty five years ago a two-years-long multicenter clinical trial started in five centers (Beirut, Paris, Roma, Sofia and Zurich) and was coordinated by the WHO. The trials were on the treatment of human cystic echinococcosis with benzimidazole carboxamides. During the period 1979 – 2014 two hundred and fifty two patients with liver echinococcosis were treated with albendazole (10 mg/kg/day for four courses of 30 days) and followed up. Forty four of the patients received mebendazole (30-50 mg/kg/day for 3 to 6 months). The treatment response was based on objective criteria provided by imaging methods – Ultrasound, Computed tomography and MRI, confirmed by serological methods (ELISA, indirect hemagglutination, immunofluorescent test, latex agglutination). The cyst changes, which characterized the cyst responses, are a criteria for determining the final chemotherapeutic effectiveness in the individual patient. Following chemotherapy, the initial change in liver cysts we observed that endocysts ruptured (detachment) at the end of 1st month therapy. The bigger hydatid cysts (<5 cm) at first increased in size, endocyst detached in 3-4th months, deformed and after 5th month started to decreased in size. The subsequent changes (hyperechoic/hyperdense appearance, size reduction), detected in all cysts in following albendazole therapy are considered as a damage of hydatid cysts. There were no serious side effects related to the applied drugs. The results of chemotherapy showed positive response of hydatid cysts observed in our patients, with multiple, recurrent, multiorgan echinococcosis and with single cysts. Chemotherapy is an alternative for patients with echinococcosis and the compelling facts prove that this method of treatment is successful.

Keywords: liver cystic echinococcosis, chemotherapy, albendazole

POSTERS

The study of preparation and relative bioavailability and liver targeting properties in rats for albendazole nano-liposome

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Background: The albendazole liposome (L-ABZ) as a hospital preparation of First Affiliated Hospital of Xinjiang Medical University has been used for clinical to treat echinococcosis effectively for 10 years, but its bioavailability and targeting properties still need to be further improved.

Objectives: To prepare the albendazole nano-liposome (NL-ABZ) based on the L-ABZ, in order to further improve the relative bioavailability ($F_{rel}$) and liver targeting properties and lay a foundation for the development of new preparation.

Material and Methods: By laser scattering method and glucan gel column chromatography, the particle size and entrapment efficiency of NL-ABZ were determined, respectively. Both of particle size and entrapment efficiency were as the evaluation indexes of high pressure homogeneous for preparation NL-ABZ, whose technology was studied. The high performance liquid chromatography (HPLC) was used to determinate the concentration of albendazole (ABZ) and its metabolites in blood and liver of rats. The $F_{rel}$ and the liver targeting properties of NL-ABZ in rats were evaluated by using the reference preparation of the albendazole tablet (T-ABZ) and L-ABZ.

Results: The laser scattering method and glucan gel column chromatography for determining the particle diameter and entrapment efficiency of NL-ABZ were established and the experimental conditions such as pressure, cycle time and temperature for preparing NL-ABZ by high pressure homogeneous technology were determined. And the particle diameter and entrapment efficiency of the NL-ABZ prepared by this method were 207.65±2.63 nm and 94.96±1.24 %, respectively. The method of using HPLC to determinate the concentration of ABZ and its metabolites in blood and liver of rats was established. Taking the albendazole sulfoxide as the detection index and T-ABZ as the reference preparation, the $F_{rel}$ of L-ABZ and NL-ABZ were 171.95% and 235.14%. Taking L-ABZ as the reference preparation, the $F_{rel}$ of NL-ABZ was 136.75%. Taking the albendazole sulfoxide as the detection index and T-ABZ as the reference preparation, the $F_{rel}$ of L-ABZ and NL-ABZ were 171.95% and 235.14%. Taking L-ABZ as the reference preparation, the $F_{rel}$ of NL-ABZ was 136.75%. Taking T-ABZ as the reference preparation, the liver targeting index (TT) of L-ABZ and NL-ABZ were 1.42 and 1.79.

Conclusion: The particle diameter, distribution and entrapment efficiency of the NL-ABZ prepared by high pressure homogeneous technology are small, uniform and high. And the animal experiments show that the $F_{rel}$ and liver targeting properties of the NL-ABZ are better than that of the L-ABZ, which provided some experimental basis for new preparation development.
The effect on the egrps9 gene expression and the oxidative stress mechanism of artesunate

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Background: Anti-malaria drugs artemisinin and its derivatives have medicinal value in the treatment of cystic echinococcosis (CE). In our preliminary work, we found that Echinococcus granulosus (E. granulosus) RPS9 (EgRPS9) was up-regulated when the protoscoleces (PSCs) of E. granulosus were treated with artemisinin (ART) and albendazole (ABZ). The purpose of this study was to detect the RPS9 gene expression in PSCs treated with AS in vitro, and to improve the mechanism of artesunate (AS) anti-echinococcosis.

Methods: EgRPS9 gene was cloned from the cDNA of PSCs and its sequence was analyzed by biology informatics. The gene expression pattern of EgRPS9 gene at different developmental stages of PSCs and drug intervention groups were detected by quantitative real-time PCR (qRT-PCR) assay. Moreover, the reactive oxygen species (ROS) content (0.5 h and 2 h) and the alkaline phosphatase activity (ALP) (12 days) of PSCs treated with DMSO, ABZ, H2O2 and AS (low and high dose) were detected, respectively. We also observed the intracellular ROS in PSCs by the confocal fluorescence microscope.

Results: EgRPS9 gene had a 554 bp open reading frame, which encoded 188 amino acids. The qRT-PCR results showed that there was no significant difference of the expression of EgRPS9 at different developmental stages of PSCs (P > 0.05). And the EgRPS9 expression was 1.27 fold in the DMSO group compared with the negative group, with no statistical difference (P > 0.05); 1.64 fold in the low dose group of artesunate compared with the DMSO group, with statistical difference (P < 0.05); 2.06, 1.65 and 1.81 fold in the H2O2 group, ABZ group and high dose group of AS compared with the DMSO group, with a significant difference (P < 0.01). Moreover, AS elevated the ROS content in PSCs in a dose-dependent manner (P < 0.01 or P < 0.05, compared with DMSO group), consistent with the confocal fluorescence microscopy results. The ALP results showed the high dose group of AS, H2O2, group and ABZ group had a rapid decrease of ALP activity within the first 6 days and kept slowly decrease within the second 6 days.

Conclusion: These findings suggest that the RPS9 induction in PSCs occurs in response to oxidative stress. This study will help us to better understand the mechanism of anti-echinococcosis activity of AS and design the next-generation anti-echinococcosis drugs against the most tenacious granulosus species.

Laparoscopic versus open approach as surgical treatment for liver hydatidosis: a systematic review and meta-analysis

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Objective: The aim of this systematic review and meta-analysis is to compare the perioperative outcomes of laparoscopic approach (LLH) with the open approach (OLH) as surgical treatment for liver hydatidosis.

Method: Electronic search of the PubMed/MEDLINE, EMBASE, Web of Science and Cochrane Library since their beginning until July 2015. We considered Randomized Control Trials (RCTs) and non-randomized comparative studies (NRCSs) comparing LLH with OLH to be eligible if they included patients with liver hydatidosis. For statistical analysis we have used Review Manager Software 5.3.5 (The Cochrane Collaboration). Mean difference (MD) was chosen as effect measure for continuous data and odds ratio (OR) for dichotomous data, reported along with the 95% confidence interval (CI). Results: There are no randomized control trials comparing open to laparoscopic approach for liver hydatid cyst. For qualitative synthesis we included 22 studies. For quantitative synthesis we included two NRCSs, 63 patients in LLH and 184 in the OLH. Conclusions: The laparoscopic approach seems to be safe and effective for hepatic hydatid disease in selected patients, with a morbidity, mortality and recurrence rate similar to open approach.

Keywords: Laparoscopy, hydatid cyst, liver, meta-analysis.

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Study of lesional aspects of cystic echinococcosis in sheep

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A morphological and structural study of over 16,500 hydatid cysts recovered from Sardinian sheep allowed to identify five different types. Unilocular cysts, 8-10% of total, show a single fluid-filled cavity and are fertile, with varying number of protoscoleces (PSC) of variable viability. Compared to the human unilocular cysts, both the laminated layer (LL) and the germinal layer (GL) of these specimens are generally thinner and microtriches along the inner surface sparser. In addition numerous large vacuoles are present in the cytoplasm of basal syncytium that appears “fenestrated”, and the proliferative cell layer is less extensive and less compact.

Multisepted cysts, mainly <1cm in diameter and among the most frequent, generally sterile, have cavity invariably divided into fluid-filled spheroidal chambers. Cyst wall is composed of a thick external adventitial and thin LL with residual GL, representing parasitic degeneration. In about 5% of the multisepted cysts “laminated brood capsules” (LBC) are recovered i.e. “anomalous” brood capsules containing an ectopic inner peripheral layer of laminated tissue in intimate contact with the internal wall surface, thought to be the likely effect of degenerative involution of the parasite (Conchedda et al, 2008. Acta Tropica 105: 215–221).

Further evolution of ovine cysts produces the Calcified type, with almost virtual internal chambers due to internal septa thickening. Very small and sterile, these cysts are the most common (>50%) and represent the final degenerative stage of parasite.

About 3-5% of cysts (frequently pulmonary) have a Caseous appearance. Similar in shape to unilocular cysts, their cavity is filled with a thick yellowish matrix of creamy consistency. Lastly, the hyperlaminated cysts (<2% of total), more frequent in the lungs, are relatively large cysts (diameter 3-5cm), the virtual cavity being filled with extensively folded and overlapping sheets of laminated tissue.

This study provided a precise understanding of how the parasite life-cycle is actually maintained and may help throw some light on the beneficial effects of a possible vaccine.

Comparison and evaluation of quality of life in hepatic cystic echinococcosis surgery patients

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Objective: Evaluation of the quality of life (QOL) in surgery patients with hepatic Cystic Echinococcosis (CE) undergoing six months, to analyze the possible influence factors and provide the scientific basis for the development of interventions. Evaluation the reliability and validity of QOL scale in hydatid surgery patients.

Methods: Evaluation the quality of life in surgery patients of hepatic CE with QOL scale. Using statistical methods such as Statistical description and Multiple stepwise regression to compare and evaluate QOL of hospitals.

Results: The results show that there are differences in physiology field, independence field, environmental field and total scores between two hospitals QOL. Hospital A’s score is higher than B in physiology field, environmental field and total scores and lower in independence field. Common factors in both hospitals are hospitalization days, previous surgery and hospitalization costs. Overall scale Cronbach’s α coefficient of QOL scale in hydatid surgery patient is 0.76, split-half reliability coefficient was 0.84. Factor analysis extracted five common factors; cumulative variance contribution rate is 80.32%.

Conclusion: According to the situation of liver CE surgery patients’ QOL, it can be providing reasonable and effective interventions for patients before and during hospitalization and after discharge. Give priority to prevent, promote the local medical, nursing, technical personnel training process, implementation of dependency treatment; doctors, nurses, volunteers trinity mode in the hospital; continuity of attention by family, community etc. out of the hospital.

Keywords: Hydatid; Quality of life; Influencing factors
Study of the mortality rate from human cystic echinococcosis and related economic impact for the period 2004 – 2013 in Bulgaria

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Aim: In 2010 cystic echinococcosis (CE) is rated as the 122nd cause of death worldwide and 90th in Bulgaria. The average mortality rate in Bulgaria during the 1959-1995 was 0.50‰000 followed by a decrease to 0.18‰000 for the 1991-2006 period. The aim of this retrospective study is to investigate mortality rate from CE in Bulgaria and to calculate unrealized gross domestic product (GDP) due to premature death caused by CE for 2004–2013 period.

Material and methods: CE associated deaths for 2004-2013 period were studied from multiple cause-coded death records in the National Statistical Institute (NSI) of Bulgaria. The mortality rate was calculated based on this information and NSI data for population size by year, gender and age groups. The economic losses were calculated from unrealized GDP for the period between the age at which death from CE occurred and 64 years of age using data on real GDP per capita in 2013 - 5,600€. The range 18-64 years of age is accepted by NSI for a period of ability to work of Bulgarian citizens.

Results: According to data of the NSI, during 2004–2013 period, 97 officially reported cases of deaths of CE in Bulgaria were found with the average mortality rate of 0.13‰000. The highest number of deaths were in 2012-14, followed by 2006 and 2008- of 12. The dynamics of mortality rate shows that it was the highest during the years 2012- 0.19‰000, 2006 and 2008- 0.16‰000 and 2013- 0.14‰000, and the lowest in 2011- 0.05‰000. The distribution of 97 dead persons by gender shows that most were male – 57 (59%) or average mortality rate 0.16‰000, compared with 40 (41%) female or average mortality rate 0.10‰000. The average age at death of CE is 54.5 years (5-93years), among them seven children have died. CE mortality rate in childhood and adolescence was 0.05‰000 and the highest is over the age 70 - 0.35‰000. For the same period, 50 people that died from CE were aged up to 64 years of age. Respectively the amount of unrealized GDP due to premature death caused by the CE of working age for 2004-2013 in Bulgaria totaled 6,619,200 €.

Conclusion: The CE is 100% preventable and that is why at this stage of development of society it is unacceptable for people to die from this disease. The indicator mortality rate and unrealized losses of GDP because of premature death can be avoided by introducing national control program including dog disinfection, reducing the number of stray dogs, vaccinating sheep and screening for early detection of asymptomatic cases.

Hydatidosis in the province of Catamarca, Argentina, anatomic location of the cysts and geographical distribution of cases in the period 2010-2014

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Introduction: the province of Catamarca is situated in the northwest of Argentina. The area of the city is 102,602 km2 and its population is about 367,820 inhabitants. Hydatidosis is an important disease in public health for the elevated losses it produces in the public health system associated to difficulties in the diagnosis of the disease and high costs of treatment. Hydatidosis is an endemic zoonosis produced by Echinococcus granulosus and it is distributed in the 16 departments of the province of Catamarca. There has been identified 3 stocks of the parasite, camel stock (G6), cow stock (G5), sheep stock (G1). The objective of this work is to present cases registered in the period 2010-2014, the anatomic location of the cysts and its geographical distribution.

Materials and methods: the cases registered correspond to patients diagnosed and derived to the department of zoonosis. In this department patients receive treatment prescribed by a doctor.

Results: 54 cases has been registered, 44 adults and 10 minors. The disease appeared in 33 women and 21 men. The ecographic study revealed the following anatomic location of the cysts: 50% in liver, 28% in lung, 7.4% of multiple cysts in liver and lung, 7.4% in spleen, 3.7% in kidney, 1.6% in abdomen and 1.8% in spinal cord. The patients diagnosed come from the 14 departments of the province. In Ambato and Antofagasta de la Sierra there has not been cases during this period, but there has existed some cases during previous years. The areas more affected are the department of Capital (17 cases) and the department of La Paz (8 cases).

Conclusion: 11 cases of hydatidosis are registered every year in the province of Catamarca. In many cases patients came from a rural area, where it is usual to give raw organs to the animals. During the last years there has been an increasing tendency of cases registered in urban areas. This situation shows the unfavorable evolution of the disease and the actual limitations of preventive and control measures. For this reason, it is necessary to improve the preventive and control measures, developing educational activities for the population: preventive measures for the public in general and strategies of early diagnosis and appropriate treatment for the health staff.

Keywords: parasite, cyst, stock.
The expression and significance of Col1al and Col3al in liver tissue next to *Echinococcus multilocularis*

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Objective: To investigate the expression of Col1al and Col3al in liver tissue of patients with alveolar hydatid infection.

Methods: 1. Subjects: Select 20 patients with liver alveolar hydatid in Hepatopancreato-biliary Surgery Department, Affiliated Hospital of Qinghai University. 2. (1) Collect the pathological specimens of operation from patients with liver alveolar hydatid; the pathological specimens were divided into group A: tissue around the lesion (according to the lesion edge 2cm); group B: normal tissue (more than 2cm from the edge of the lesion). Two groups of specimens preserved in -80°C refrigerator to spare. (2) The pathological specimens were sent to the pathology department of our hospital and made into paraffin section of 3um thick. (3) HE and Masson staining; Observe the pathological changes and fibrosis of tissue around the lesion with *Echinococcus multilocularis* infection under the light microscopy; Detect the expression and localization of Col1al and Col3al in liver tissue by immunohistochemical. (4) Disposal data and perform a statistical analysis, then draw the conclusion.

Result: The fibrosis score of adjacent normal tissue and tumor tissue was: T = 132, P < 0.01, they were statistically significant. The CRI of Col1al and Col3al in the tissue around the lesion and normal liver tissue respectively was (7.45 ± 1.85 VS 3.10 ± 1.02), (8.00 ± 1.62 VS 3.5 ± 0.89), t values respectively was -9.21, -10.88, the difference had significant statistical significance (p < 0.001).

Conclusion: There were hepatic fibrosis in tissue around the lesion of patients with alveolar hydatid infection; the main components of collagen were Col1al and Col3al in tissue around the lesion.

Keywords: *Echinococcus multilocularis*, Col1al, Col3al, Hepatic fibrosis
Cystic echinococcosis. Unusual location. Province of Catamarca, Argentina

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Cystic echinococcosis is an endemic zoonosis in Argentina. Hydatid cysts in human beings are most frequently located in the liver and lungs; other organs can be affected but to a lesser degree: kidneys, brain, heart, bones and muscles. In the years 2000-2014, the province of Catamarca registered 158 human cystic echinococcosis cases (CE). This work is based on this information and on the high goat breeding activity and organ involvement. Their ages were from 6 to 75 years. (FF-PETs).

sections from formalin-fixed paraffin-embedded tissues confirmed CE. All cysts were identified using histological parasitic material consisted of 60 cysts from 57 patients of unusual localizations of CE in the human body. The dominantly affected organs are the liver and the lungs, problem and remains endemic in Tunisia. The most prevalent indigenous parasitic disease in the country. Morbidity in 2012 was 4.37‰ and continues to hold. Moreover sporadic cases are usually diagnosed. Often epidemic foci confined to certain families - with a few infected persons in a household found. Fourteen family hydatid disease foci with a total of 35 infected persons in them registered during the last five years were presented. In diagnosing a single case other family members of patients with hydatidosis - in order to detect latent morbidity. Data indicated also the need of sanitary parasitological investigations in epidemic foci of hydatid disease to clarify the source of the infection.

Unusual sites of cystic echinococcosis: an experience from Tunisia

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Cystic echinococcosis (CE) is a worldwide public health problem and remains endemic in Tunisia. The most predominantly affected organs are the liver and the lungs, but other sites may be affected less commonly. The aim of this study was to determine the prevalence of unusual localizations of CE in the human body. The parasitic material consisted of 60 cysts from 57 patients (36 female: 65% and 21 male: 35%) with histologically confirmed CE. All cysts were identified using histological sections from formalin-fixed paraffin-embedded tissues (FF-PETs).

The patients were analyzed in terms of their age, gender and organ involvement. Their ages were from 6 to 75 years. The difference in gender’s implication in CE was statistically significant. Samples from up to 16 unusual sites were processed, including the kidney, spleen, thigh and one cyst from each of the heart, pericardial region, peritonem, sub-mandibular region, ovary, breast, gall bladder, latero-cervical region, subcutaneous tissue, vertebral column, axillary region, pleura and the abdomen. Multi-organ involvement was observed in one case. No statistical correlation was found between cyst site and either the gender or the age was observed in one case. No statistical correlation was found between cyst site and either the gender or the age...
**Underdiagnosed secondary spleen hydatidosis - case presentation**

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*Echinococcus granulosus* causes one of the many neglected parasitic infections of our planet. When the larval stage of this parasite enters the human host, cystic hydatidosis, CH, appears. The primary locations of the larvae are the liver and the lungs and from here the disease can disseminate in every tissue or organ.

In this work we present the case of a 37 year old female patient who was urgently introduced to the Surgery Unit of the Emergency Hospital Tg. Mureș because of diffuse abdominal pain in the superior, epigastric, left hypochondrial quadrants which were radiating to the left dorsal segment. The woman also accused loss of appetite, weight loss, lipothymia, astenia. In 2012 she underwent a cholecystectomy for chronic cholecystitis.

During the laparoscopic intervention a hydatid cyst was diagnosed in the second segment of the liver which was removed. As a complication of the case, the cyst was perforated and daughter cysts were disseminated in the peritoneal area. The recommended postoperative treatment with Albendazol was ceased because of intolerance against it. The patient suffered from other diseases, such as epilepsy with generalized tonic crises, intolerance against lactose, endometriosis and has been hospitalized for many times. Laboratory findings, specific IgG antibody detection against *Echinococcus granulosus* and *Echinococcus multilocularis* were negative. No eosinophilia was observed. Abdominal CT scan showed on the lower part of the spleen a well-defined septated hypodense formation, with liquid inside. After the premedication, general anesthesia was performed and median xypho-subumbilical laparoscopy revealed a cca 4x3 cm tumour on the inferior pole of the spleen, with adhesions to the diaphragm. The spleen was completely removed and the patient’s evolution after the operation was satisfactory.

The conclusion of the case is that in many cases CH is discovered accidentally, symptoms sometimes are missing. When a hydatid cyst is removed, it has to be performed in such a manner that spreading of the disease is prevented. The postoperative follow-up must continue for months, even years, because the medical staff needs to be aware of secondary dissemination of the disease.

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**Genotyping and phylogenetic analysis of cystic echinococcosis isolated from animals and humans in Aegean Region, Turkey**

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**Introduction:** Cystic echinococcosis (CE) still remains an important global health and economic problem in endemic countries such as Turkey. Exposure of humans to CE is common in Turkey as the majority of people living in rural areas is engaged in animal husbandry. Limited reports on the strain characteristics of *Echinococcus granulosus* in Turkey have been published until now.

**Aim:** The aim of this study is to investigate identification of *E. granulosus* strains infecting animals and humans in the Aegean Region of Turkey.

**Material and Methods:** Totally, 60 isolates of hydatid cyst were collected from humans and animals. Thirty two animal samples were collected from the liver and lungs of cattle and sheep and 28 human isolates were obtained from patients who underwent surgery at Ege University Hospital and Celal Bayar University Hospital between 2010 and 2014. Fertility and the viability of the cysts were observed microscopically. The total genomic DNA was extracted using RTA-DNA Isolation Kit (Gebze / Kocaeli, Turkey) according to manufacturer’s instructions.

**Results:** After the PCR, to investigate the genetic characteristics of isolates, ribosomal ITS-1 gene region was digested with restriction endonucleases and deoxyribonucleic acid sequencing of the mitochondrial COX1 and NADH 1 genes were performed with ABI Prism Genetic Analyzer 310 instrument. Forward and reverse sequences of amplicons were examined with Sequencing Analysis software and then their alignment analyze were done by using SeqScape V2.6 software. After the alignment analyze all samples were examined with NCBI BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare other *E. granulosus* sequence data. Phylogenetic analysis of strains was done by Geneious software. As a result of our study all cattle, sheep and human isolates were detected as *E.granulosus* s.s. (G1-G3 complex) with few nucleotide differences.

**Conclusion:** The results showed that the *E.granulosus* s.s. is the predominant species in the Aegean Region of Turkey even G7 strain was determined in one of the study which was published before. Identification of large number of *E.granulosus* DNA isolates from animal and human in different regions of Turkey is important to establish a hydatid control program.

This investigation was supported by Celal Bayar University Scientific Research Projects Foundation Unit.

**Keywords:** *Echinococcus granulosus*, Genotyping, Phylogenetic analysis, Aegean Region, Turkey
Introduction: Cystic Echinococcosis (CE) is caused by the metacestode of *Echinococcus granulosus*. The metacestode can be classified as either fertile or infertile according to their capability to produce protoscoleces. Our previous work showed different IgG subclasses (IgGS) within the germinal layer of both fertile and infertile bovine cysts, however serum IgG5 levels and the co-infection with other parasitic diseases such as *Fasciola hepatica* (FH) were not considered.

Material and Methods: Bovine blood and tissue samples were collected in abattoirs in Santiago, Chile. Liver and lungs were inspected to assess the presence of FH and CE. A commercial ELISA Kit was used to confirm serological FH infection, classifying the samples in 6 categories according to presence (+) or absence (-) of FH and CE, and CE fertility. Sera IgG5 were analyzed using the Bethyl Laboratories™ Bovine IgG1 and IgG2 ELISA Quantitation Sets. Immunohistochemistry assay was performed using the same Bethyl Laboratories™ HRP conjugated antibodies.

Results: The FH commercial ELISA kit validity was assessed, finding 97.5% sensitivity and 87.6% specificity. FH serological prevalence in CE infected animals was 52.4% (76/145), 48.4% in infertile (67/132) and 69.2% in fertile (9/13) CE. FH(-)CE(-) animals had a sera IgG1/IgG2 mean ratio of 0.7. Fertile and Infertile infected CE(+)FH(+) had IgG1/IgG2 ratios of 0.5 and 0.57, respectively. FH infected animals had an IgG1/IgG2 ratio of 0.81; 0.84 in infertile CE increasing to 1.03 in fertile CE infections, the latest showing the highest ratio compared all the other groups (p<0.05). Sera IgG1/IgG2 ratio levels were related to IgG1 and IgG2 lung tissue reactivity were FH(-)EC(-) showed a higher IgG1 over IgG2 reactivity, the latest increasing in the presence of FH infection. In fertile CE infections IgG1 signal seems slightly higher than IgG2. In infertile CE infection alone, IgG2 shows stronger reactivity than IgG1 opposite of the IgG1/IgG2 signal shown with concomitant FH infection.

Discussion: Healthy animals had a 0.7 IgG1/IgG2 median ratio, which dropped with CE infection; FH co-infection seems to modulate the immune response to a higher ratio, especially in fertile CE infection, that could be indicating a higher production of specific type of IgGs. IgG subclasses present in the tissue samples showed a similar tendency than the sera, except in infertile CE with concomitant FH infection. Grant from FONDECYT N° 1130717

**Echinococcus granulosus – Immunoprotection in a murine model of secondary hydatidosis following immunization with a cocktail of EG95 DNA prime and protein boost**

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Aim: Cystic Echinococcosis/hydatidosis (CE) is one of the most serious emerging zoonotic parasitic diseases throughout the world caused by the larval stage of *Echinococcus granulosus*. Vaccination of livestock may provide an additional approach to the control of echinococcosis. This study aimed to investigate the immunoprotection of the recombinant cocktail of EG95 DNA prime and protein boost in a murine model.

Material and Methods: RNA was extracted from oncosphers and protoscoleces then amplified by PCR and RT-PCR with specific primers of EG95. Afterward the purified RT-PCR products were successfully ligated into pJET1.2 plasmid vector. The pcDNA3 plasmid was used as expression vector and Eg95 fragment sub cloned into this plasmid. The recombinant plasmid pET32-a+ was used for Eg95 expression. The purified recombinant proteins were confirmed by Western blot analysis using polyclonal antiserum. Three groups of female mice were immunized subcutaneously with pcDNA3-Eg95 construction once and two protein boost, then the mice were challenged with *Echinococcus granulosus* protoscoleces (PSCs) intraperitoneally. Finally, the humoral immune and the cellular immune responses were evaluated.

Results: Mice immunized with the cocktail of DNA prime and protein boost produced effective immunity protection (reductions in cyst load more than 96.11%). The immunized mice showed specific IgG, IgG1 and IgG2a responses accompanied by IFN-γ significantly increasing after immunization. Nevertheless, the level of IL-4 and IL-10 increased in the control group compared to the test groups.

Conclusion: Our findings suggest a new construct of DNA vaccine candidate that can elicit a strong cellular and humoral response against cystic echinococcosis/hydatidosis.
The diagnostic value of an immunochromatographic test in cystic echinococcosis in human

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Aim: Cystic echinococcosis (CE) is a disease of mainly sheep, cattle, swine and goats etc. The infection is transmitted to humans by ingesting *E. granulosus* eggs released in the faeces of definitive hosts such as dogs. The larval stage of parasite develops a slowly enlarging cyst in visceral organs mainly liver and/or lung. CE may be considered as an important public health problem in Turkey, because of causing morbidity and economic loses. The aim of the present study was to evaluate diagnostic value of an immunochromatographic test (ICT) for CE.

Methods: Total 50 sera from surgically and/or pathologically confirmed CE patients were included in the study as study group; control group was the patients who were ELISA negative. Sera were selected from collection at Adnan Menderes University, Faculty of Medicine, Parasitology Laboratory by simple random sampling. The collection was composed of sera collected between 2010 and 2014, antibody titers of each serum was determined with in-house ELISA before storage at -20°C. The presence of *E. granulosus* antibody in sera was determined with a commercially available ICT (VIRapid® HYDATIDOSIS) and in-house Western Blotting methods.

Results: In the study group (*E. granulosus* confirmed cases), two (4%) of the 50 sera were negative with ICT, 48 (96%) were positive. In the control group (ELISA negative) all were negative with ICT. We found nine antigenic bands with molecular weights of 8 kDa to 110 kDa in study group with WB analysis; the number of bands and densities varied according to the antibody titers of sera. Moreover, no antigenic band was detected in sera of control group. The sensitivity and selectivity of ICT was %96 and %100, respectively, if WB accepted as golden standard.

Conclusion: It may be concluded that ICT has significant advantages because it allows testing without any equipment; the method is fast and more individuals can be studied in short time. The current study highlights the importance of ICT; however, further studies are needed regarding the possible cross reactions in extended series of cases.

Optimization of freeze-drying process for anti-hydatid drug albendazole nanosuspension

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Objective: Increasing the solubility of albendazole is a key factor of the drug treatment of echinococcosis. The albendazole nanosuspension prepared by liquid precipitation method solves the solubility problem well, but, the stability of nanosuspension is not satisfied. The purpose of this research is to improve the stability of albendazole nanosuspension through optimizing the freeze-drying process of nanosuspension.

Material and Methods: With albendazole API as raw materials, the liquid precipitation method was adopted to prepare albendazole nanosuspension, from which albendazole nanometer powder was made through the freeze-drying technique. With the particle size, zeta-potential and polydispersity index as the indicators, the monofactor method was applied to investigate and identify the main factors effecting freeze-drying technique, such as category, ratio and concentration of cryoprotectant and pre-freezing temperature. And therefore, the optimal freeze-drying technique was established.

Results: The optimized freeze-drying process conditions after screening were as follows: the pre-freezing temperature was -20°C, the cryoprotectant was 70:30 (v/v) mixture of mannitol and glucose and the concentration of cryoprotectant was 4%. Under these conditions, the size of nanometer powder prepared was (205.70±2.06) nm and the Zeta potential was (-15.53±0.18) mv, and the powder was well-distributed, indicating that the freeze-drying effect was excellent.

Conclusions: The optimized freeze-drying technique for albendazole nanosuspension can improve the stability of albendazole nanosuspension, meanwhile maintaining its original nano-characters.
Genotyping of hydatid cyst isolated from human in Ilam province, West Iran

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Introduction: Usually in areas where cystic echinococcosis is endemic, there is a relatively high genetic diversity in the parasite biology. The aim of present study was to determine genotype of human isolates of hydatid cyst in Ilam province, West Iran.

Materials & methods: 10 human hydatid cyst samples were collected from Ilam hospitals. After DNA extraction of protoscoleces, mitDNA NADH dehydrogenase subunit 1 (nad-1) region was amplified by PCR and the PCR products were analyzed by PCR-RFLP.

Results: Based on the results obtained, the size of proliferative nad-1 products was 550 base pairs. Patterns of parts which obtained from PCR products after cutting by AluI and RsaI enzymes showed that all samples had a similar RFLP pattern but HpaII enzyme did not cut any region.

Conclusion: The results of this study indicated that there is at least one genotype of parasite in this region which belongs to (G1-G3) complex.

Keywords: Echinococcus granulosus, genotypes, PCR-RFLP, Ilam.

Molecular Characterization of Cystic Echinococcosis Isolated from Human Samples in Aegean Region, Turkey

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Introduction: Human cystic echinococcus (CE) is a severe disease caused by the larval metacestode stage of the zoonotic Echinococcus granulosus. This larval infection is still one of the most important problems for issues relating to public health and the economy is affecting an estimated 2-3 million people in the world. At least ten genotypic defined strains (G1–G10) which were examined within the E. granulosus complex, are important control programs may influence life-cycle patterns, antigenicity, host specificity, sensitivity to chemotherapeutic agents, transmission dynamics.

Aim: The present study was aimed to investigate molecular characterization of Echinococcus granulosus isolates which were collected from 10 human hydatid fluid samples treated with PAIR procedure. These samples were analyzed by PCR of mitochondrial genes COX1, NADH1 and RFLP analysis of ribosomal ITS-1 gene region.

Material and Methods: In this study, 10 individual hydatid cyst samples were isolated from the liver of humans who were attended to Ege University Hospital and were examined for their fertility and viability under light microscope. The total genomic DNA was extracted from protoscoleces by using Qiagen DNeasy Isolation Kit according to manufacturer instructions with few differences. Quantification and qualification of total genomic DNA was measured by Nanodrop ND-1000 spectrophotometer. To investigate the genetic characteristics of isolates, mitochondrial COX1 and NADH 1 genes and ribosomal ITS-1 gene region were amplified by PCR. COX1, NADH1 and ITS-1 genes’ PCR products were separated with 1.5% agarose gel electrophoresis. After the PCR, ITS-1 amplicons were digested with Rsal,MspI,CfoI and AluI restriction enzymes.

Results: All samples and genes were shown same band patterns even after restriction enzymes digestion. As a result of our study all human (both males and females) isolates were detected as E. granulosus sensu stricto (G1-G3 complex).

Conclusion: The results showed that the E. granulosus s.s. remains the most widespread genotype infecting humans in the Aegean Region of Turkey even G7 strain was determined in one of the study was published before. Molecular characterization of E. granulosus strains is very important for CE treatment and establishing control program in Turkey.

Keywords: Echinococcus granulosus, Genotyping, RFLP, Aegean Region, Turkey

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Isolation, enrichment and initial proteomic characterization of exosomes inside sheep hydatid cysts

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Aims: The larval stage of the parasite Echinococcus granulosus can develop and form fertile and non-fertile hydatid cysts in its intermediate hosts causing cystic echinococcosis, a neglected parasitic disease. The parasite controls local inflammation at the initial phases of infection for the establishment and survival of the hydatid cyst. In this sense, the delivery of exosomes by helminths and their internalization by host cells has been recently described. Many extracellular parasites secrete microvesicles containing molecules that regulate host gene expression and immunity. Specifically, exosomes are 30-100 nm membrane vesicles of endocytic origin produced by different types of cells involved in promoting inter-cellular communication and antigen presentation. The characterization of exosomal cargo of parasites might provide new biomarkers for disease diagnosis, prognosis and response to treatment.

Material and methods: We have isolated microvesicles from fertile and non-fertile sheep cysts by ultracentrifugation and obtained a fraction further enriched in exosomes by optiprep density gradient. This fraction has been characterized by NanoSight and cryo-electron microscopy. Exosomes from fertile and non-fertile cysts have been lysed and their proteins resolved in SDS-PAGE gels and silver stained separately. Ten gel bands from both cyst types have been used for LC-MSMS qualitative analysis and identifications using MASCOT algorithm in three technical replicates.

Results: Exosomes have been isolated from hydatid cysts. The Gene Ontology analysis of the identified proteins demonstrates the presence of exosomes of both parasite and host origin inside sheep cysts and the presence of specific parasite molecules in the exosomal cargo.

Conclusion: The identified proteins highlight the nature of the vesicles providing the first data about the protein cargo in hydatid cyst exosomes. This information could be very useful for subsequent experiments to identify new biomarkers and targets for the clinical management of this disease.

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Identification and genetic characterization of hydatid cyst in a wild boar, in Turkey

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Cystic echinococcosis is one of the major zoonotic, endemic diseases in Turkey. It induces public health problem and economic losses in animal instructor. Many studies have been performed about prevalence of this disease in sheep, goat, cattle, water buffalo etc. (10,2-74,4%) and also genetic characterizations of hydatid cysts in recent studies, too. G1 (human, sheep, cattle, water buffalo), G3 (sheep, cattle, human), G1-G3 complex (horse), G6 (human) and G7 (human, sheep) strains were detected in Turkey.

In the present investigation, we described the first molecular analysis of E. granulosus infecting wild boar in Turkey.

Cysts samples were obtained from a 9 year old, female wild boar. There were a lot of fluctuant individual cysts on the liver, lungs, spleen, kidney and body cavity. Cysts were measured; 1-10,5 x 1-15 cm external diameter. Hydatid cysts identified non calcified, non caseous and were sterile.

The tissue specimens were fixed in formalin 10%, processed routinely, embedded in paraffin. Paraffin blocks were sectioned at 5 µm and stained with Haematoxylin-Eosin (H&E) for pathological examination. After these findings cirrhosis parasitaria and subacute nephritis are diagnosed.

Genomic DNA was extracted from germinal layers of individual hydatid cysts. It was then purified using the Gene Jet Genomic DNA Purification Kit (Thermoscientific, Lithuania) following the manufacturer’s instructions.

PCR was performed a mitochondrial DNA the cytochrome c oxidase subunit I (cox 1) gene, using JB3/JB4.5 primers as reported by Bowles et al. (1992).

The positive PCR products were purified using a commercial kit for sequencing. The sequenced amplicons of mtDNA cox I were aligned with other mtDNA cox I sequences from GenBank using BioEdit software, a phylogenetic tree was constructed from these aligned sequences using MEGA (version 6.0).

The isolates identified as Echinococcus granulosus strain after sequenced but, Turkey’s and other countries sequenced were compared, there is a separe structure on these samples.

References: The research that led to these results has received funding from the FP7 (HERACLES) g.a. 602051.
The genotype distribution of human E. granulosus isolates in Aydin, Turkey

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Aim: Echinococcus granulosus (E. granulosus), the etiological agent of Cystic Echinococcosis (CE) in humans and livestock, is a widely distributed zoonotic pathogen tapeworm that causes significant morbidity and economic losses in many countries, and also in Turkey. A high level of genetic diversity was reported between the isolates of E. granulosus. Up to now, ten genotypes (G1-10) of E. granulosus have been identified from different hosts by molecular genetic analysis. In most of the studies from Turkey and all around the world, G1 (sheep strain) was reported as the prevalent genotype in human and other hosts. Additionally, G3, G6 and G7 were also reported from Turkey. However, we still have limited information about the genotypes of human E. granulosus in Turkey. The aim of the present study was to determine genotypes of E. granulosus isolates in Aydin, Turkey.

Methods: The study was conducted in Adnan Menderes University, Parasitology Laboratory. Genomic DNA was isolated with a commercially available kit (QIAGEN DNA Mini Kit, Germany) from cyst fluids, aspirated during surgery. Mitochondrial, cytochrome oxidase subunit I (cox1) gene, was amplified with a single round PCR and sequenced. The sequences were analyzed by using BLAST tool on website of NCBI. Genotypes were determined according to closest or exact matches in comparison with previously deposited sequences in Genbank. Additionally, a phylogenetic tree was constructed by using Neighbour Joining Tree.

Results: A total of 20 isolates were successfully amplified and sequences were acquired. Sheep strain G1 was accounted the greatest majority of our isolates (13 isolates, 61.9%). The other genotype was G7 (4 isolates 19%) which is pig originated genotype and one isolate (4.7%) was identified as G1-3 cluster. However, two of sequences (10%) showed little similarity with E. granulosus cox1 gene (58-67%) and they were eliminated.

Conclusions: Our study confirms the previous findings in Turkey that have indicated the predominance of sheep strain (G1). Additionally, similar to our findings pig strain was reported less than sheep strain in previous studies from Turkey. It may be concluded that sheep and wild boars in the study area may be the main agent of human CE cases in study area. The research has also shown that cox1 gene is a valuable target for determination of E. granulosus genotypes.

Availability, cyst characteristics and hook morphology of Echinococcus granulosus isolates from livestock (cattle, sheep and goats) in Pakistan

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Cystic Echinococcosis (CE) is a zoonotic infection caused by larval (metacestode) stages of cestodes belonging to the genus Echinococcus and the family Taeniidae. The aim of current study was to determine the availability and organ placement of hydatid cysts in some ruminants of Pakistan and for the study of rostellar hooks morphology of protoscoleces of E. granulosus. A total of 2803 animals comprising 925 sheep, 939 goats and 939 cattle (n=) from both sexes were examined to find out the prevalence of hydatid cysts in different regions of Central Punjab from January to December 2013. The overall prevalence of hydatidosis was determined as 3.24%, 2.44% and 2.44% in examined sheep, goats and cattle, respectively. The localization of hydatid cysts in the livers of infected sheep, goats and cattle was found as 1.4%, 1.17% and 1.17%, respectively while lung localizations were determined as 1.83%, 1.27% and 1.27% in the same order. Among the 33, 25 and 30 examined hydatid cysts in sheep, goats and cattle, 8 (24.2%), 11 (33.3%), 8 (24.2%), 6 (18.1%), 9 (36%), 9 (36%), 4 (16%), 3 (12%) and 10 (33.3%), 11 (36.6%), 4 (13.3%), 5 (16.6%) were characterized as fertile, sterile, calcified and under-developed, respectively. The total number of hooks on protoscoleces was 28.40±1.72 (sheep origin), 21.0±1.06 (goat origin) and 27.70±1.11 (cattle origin). In conclusion our investigation revealed that availability of hydatid cysts is still significantly higher among all examined livestock.
Epidemiological survey on Echinococcosis in Banma County, Guoluo Tibetan Autonomous Prefecture of Qinghai Province

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Objective: To assess the epidemiological actuality of echinococcosis in Banma County, Guoluo Tibetan Autonomous Prefecture of Qinghai Province.

Method: All participants were recruited by random Cluster Sampling. The participants were screened by B ultrasound and interviewed by a paper questionnaire.

Results: 1801 people were recruited and examined by B ultrasound. 217 were diagnosed as hydatid disease with an overall prevalence of 12.05%, among which 2.61% were CE and 9.44% were AE. The prevalence of Echinococcosis in different professional subgroups were statistical difference ($\chi^2$=13.097, P<0.001), among which the prevalence in herdman was highest (19.91%). Meanwhile, the prevalence in different regions also showed a significant difference ($\chi^2$=35.935, P<0.001) and the Daka township was the most prevalent, with a prevalence of 18.04%. The prevalence of Echinococcosis was highest 50- subgroup, with a prevalence of 28.57%, and its distribution has statistical difference in age-subgroups ($\chi^2$=90.608, P<0.001).

Conclusion: AE and CE were prevalent in Banma County of Qinghai Province. The female, herders, elder (50–) and resident in Daka township were high risk groups. The prevention and control solution of echinococcosis should be more proactive in future.

Keywords: Echinococcosis; Epidemiology prevalence; Banma County

Hydatid cysts with rare localizations – diagnosis and therapeutical approach

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Aim: Hydatid cysts still represent an important number of cases in the pediatric surgical pathology. Apart from the usual localizations - liver and lungs - other localizations, today seldom encountered, represent a real diagnostic challenge. It may even represent an intraoperative finding which determines the surgeon to change the initial therapeutic approach. This paper is a retrospective study which aims to present our experience over a 30 years period regarding rare localizations of hydatid cysts.

Materials and Method: The paper analyses 462 cases of children, out of which 28 presented with unusual localizations of Echinococcus granulosus infection surgically treated in our department between 1984 and 2014. The clinical presentation, preoperative diagnostic tests, treatment strategy and postoperative results are presented.

Results: Four-hundred and sixty-two patients with hydatid cysts were treated during the mentioned period. Twenty-eight of them were found to have lesions in less usual locations: brain (4), pancreas (3), spleen (6), kidneys (6), broad ligaments of the uterus (5) and intramuscular (4). In 8 cases, multiple synchronous cyst localizations were found: 3 cases with cerebral and liver cysts, 2 cases with pulmonary and broad ligament of the uterus cysts, 2 cases with hepatic and muscle cysts, 1 case with cerebral, pulmonary and hepatic cysts. In 6 cases we performed cysts excision and on the remaining cases cystotomy with the removal of the proligerous membrane, followed by drainage was the therapeutic method applied. There were no mortalities and no recurrences in the 2 to 13 years of follow-up.

Conclusions: Hydatid cysts, even today, remain an important part of the differential diagnosis of any cysts either in the liver, lungs or in any other location. Synchronous multiple cysts must always be considered a possibility and investigated accordingly. The therapeutic approach does not raise special problems due to the tissue elasticity specific to children and usually, the remaining cavity is not a source of complications as in adults.
An overview of Cystic Echinococcosis in Sardinia

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Cystic Echinococcosis (CE) remains a major, though often underestimated, public health problem in many European areas as in Sardinia, an Italian island in the central Mediterranean Basin. Notwithstanding the discontinuous efforts and investments expended over the years with 3 tentative control attempts from the 1960s to the 1990s, the zoonosis still represents a serious health problem in the island. Human incidence rates, as high as 14.32 per 100,000 inhabitants in the 1940-50s ranged, over a thirty year period, from 14.6 per 100,000 inhabitants in 1969-1973 to 9.8 in 1990-1995, to 6.62 in the last survey (Conchedda et al, 2010. Parasitol. Int. 59: 454-459). The costs of disease are relevant, considering that value of hospitalization alone is estimated at around 4 million Euros for the period 2001-2005. Demographic and socioeconomic changes over the last few decades have modified diffusion scenarios and created the conditions for an unevenly distributed reduction. The areas with the highest incidence of human CE coincide with the main traditional sheep-rearing areas and those where the prevalence in sheep is highest. In the island prevalence in sheep, the most relevant intermediate host, varied from 77-80% in the 1950-60s, to 83-88% in 1979-1989, immediately before the last (interrupted) control attempt. More recent studies, carried out during 1995-2010, i.e. 10 years after the last campaign, showed this figure to have diminished to about 75% in northern Sardinia (Scala et al, 2006. Vet. Parasitol. 15 (1): 33-38), 82.6% in the most pastoral province. In Southern Sardinia prevalence has decreased to about 65% (Conchedda et al, 2012. Acta Tropica 122: 52-58), peaking at 78% in the most rural province against 58% in the most “urbanized”, where the reduction was greatest. Notwithstanding the decreasing trend in parasite pressure, the persistence of CE in Sardinia, especially in less favored areas and the related costs, confirm the need for specific control measures, particularly considering the vaccine option.

Interaction with Municipalities in the Zoonosis Control

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The National Commission of Zoonosis of Uruguay (CNZ) is the decentralized government agency that coordinates inter-agency efforts on surveillance and control of zoonotic diseases, particularly Cystic echinococcosis. Uruguay has a political organization of 19 departments in which there are around 350,000 people living in risky areas to catch Hidatidosis (RA).

From 2013 to 2015 CNZ signed agreements with all the departmental governments. The following actions are strengthened in every department: a) Decentralization and active participation of the institutions and the community. b) Massive campaign of free sterilization on dogs from rural and suburban areas (over 200,000). c) Ultrasound diagnosis of liver hydatid disease in people from RA d) Formal and informal education. These actions are performed on mobile clinician consulting room traveling in rural schools and neighborhood clinics.

Results: a) Dog population stabilization, confirmed by surveys (Equipos Consultores Uruguay) in which the majority of the population be aware of less or equal number of dogs (57%). b) In more than 200,000 ultrasound screening was found that 1-2% are carriers of liver hydatid cyst in RA (it is around 7000-10000 people who have the disease but do not know). c) After public inquests (Equipos Consultores, in April 2014 with a margin of error of +/-2.8%) was found: c.1) the image of the actions of the CNZ is very positive (85%); c.2) a high percentage of the country’s population (67%) know the disease and its transmission mode (should be) but do not apply this intrinsic value.

Conclusion: The departmental governments are the most important political and executive organ to perform these activities with the community, reducing the gap between the “should be” and its application.
Cystic echinococcosis is endemic in the Rio Negro province of Argentina and, for this reason; a control program using praziquantel in dogs was developed from 1980. The transmission rate to humans and sheep has decreased significantly, however transmission persists. In 2009 the vaccination of sheep with EG95 was incorporated in some areas of the province. The objective of the study was to evaluate the humoral responses to the vaccine EG95. Lambs received two vaccinations with the EG95 vaccine followed by a single booster injection when the animals were 1.5 years of age. Blood samples from vaccinated animals were obtained for determination of antibody titers against EG95 protein. Anti-EG95 responses were determined as described by Heath and Koolaard, 2012. Blood samples were centrifuged to obtain serum and samples were maintained at 5°C before prior to being stored at -20°C. Responses were evaluated from a total of 189 animals (sheep of different ages that were all bled in 2014, no vaccinated, old sheep for control area, lamb with first doses only just before second dose, lamb with second doses just before the third doses, sheep with the three doses one year after doses 3, sheep with three doses two year after doses 3, sheep with three doses three years after doses 3, sheep with three doses four years after doses 3). Responses after the third vaccination at 1.5 year of age induce an increase in titer greater than seen following two immunizations. In the third and fourth years of the study there was a decrease in the level of antibody response coinciding with the period following eruption of the Puyehue volcano in Chile. The study shows the dispersion range of the individual values of the samples in the first group vaccinated from which only 1 had a titer less that the average of the animals without vaccination. Humoral response to immunization, under field conditions, has proved consistent with experimental studies, increasing with the application of the second dose and then peaking reinforcement year. The lower optical density values were observed in two cohorts of vaccinated animals suffering greater nutritional difficulties, although the differences were not significant.

**Keywords:** echinococcosis, vaccine EG95, sheep, antibodies

Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: second study of impact

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Cystic echinococcosis is endemic in the Rio Negro province of Argentina. After 30 years of control using praziquantel in dogs the transmission rate to humans and sheep has decreased significantly, however transmission persists. The objective of the study was to assess the impact of the inclusion of the EG95 vaccine for sheep in the control program. The vaccine was applied in an area comprising communities of native people. Two different groups were assigned to different treatment types. One group comprising 71 farmers of Blancura Centro and Lipetren regions was established as the control region where no vaccinations were undertaken. In the treatment group, comprising 79 farmers of Anecón Grande, Manuel Chiquique, Nahuel Pan and Rio Chico abajo regions, lambs received two vaccinations with the EG95 vaccine followed by a single booster injection when the animals were 1-1.5 years of age. The prevalence of CE in sheep in vaccinated area at the start of the trial (2009) was determined by ELISA/Westernblot as being 26.2% in 2-4 tooth animals. In 2011 7.8% of the 2-4 tooth age class were positive (p=0.006 between 2009 and 2011). In relation with the farmers in 2009 80.0% had at least one animal positive to ELISA/Westernblot in the 2-4 tooth age class and 42.8% in 2011. Evidence for *Echinococcus granulosus* transmission was monitored by necropsy on adult sheep in 2015. A total of 21443 doses of EG95 vaccine were applied in the period 2009-2015. Before the vaccine was introduced, 56.3% of 6-year-old animals were positive at necropsy. The prevalence decreased to 21.1% in 6-year old animals following use of the vaccine. The number of cysts per infected animal also decreased from 1.4 to 0.3. Despite the difficulties of implementing vaccination in this region of Rio Negro and 47% of the animals being fully vaccinated in the mong those that were assessed by necropsy, the EG95 vaccine achieved a 62.5% reduction in infection in 6 year old animals. Nevertheless, this trial has demonstrated the usefulness of the EG95 vaccine in a valuable tool to assist with reducing *E. granulosus* transmission, even in circumstances where delivery of the program faces many practical difficulties. In the future it will be important to demonstrate the effect of sheep vaccination on transmission to dogs and hence the likely effects on transmission of CE to humans.

**Keywords:** echinococcosis, vaccine EG95, sheep, control, prevalence
**Echinococcus granulosus** egg dissemination: role of slaughterhouses in the spread of the parasite

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Hydatidosis is a zoonosis which has become a real concern for health care institutions and animal rearers in Tunisia. The Tunisian endemicity is aggravated by the growing number of dogs and the difficulty of getting rid of the contaminated viscera because of the lack of equipment in most of slaughterhouses. Considering aspects related to public and animal health, the aim of this study is to evaluate the role of the slaughterhouses in the canine infection and **E. granulosus** eggs dissemination.

A total of 553 dog faecal samples were collected from the ground in six Tunisian regions (Monastir, Sousse, Zarzis, Medenouf, Douz and Tataouine). 219 isolates were taken around the slaughterhouses and 334 from places distant from them (10-15 km). Helminth eggs were concentrated by the use of sucrose gradient flotation. The presence of the taeniid eggs was assessed using light microscope examination. The presence of **E. granulosus** eggs was ascertained by the specific **EgG1**III PCR. The contamination index (percentage of dog faeces positive for **E. granulosus** eggs) from isolates collected in the different regions was analyzed with the Pearson's Chi-squared Test, using the SPSS software version 18.0. The level of significance was set at p<0.05. The difference between the parasite egg prevalence was calculated at the 95% confidence interval (95% CI).

Depending on the six studied regions the contamination index varied from 10.5% to 42.3%. It ranged from 0% to 42.5% and from 0% to 46.5% in sampling sites around abattoirs and distant from them respectively. No relationship between the studied regions and the contamination index distribution was observed by using the Chi-squared test (p=0.26). The average contamination index from the regions around slaughterhouses (23.3%, 95% CI: 17.7-28.9%) was in the same range as that detected in squares located far from slaughterhouses (26.0%, 95% CI: 21.3–30.8%).

The Chi-squared test confirmed that the contamination index distribution was independent from the presence or absence of slaughterhouses (p=0.51).

The pathogen dissemination is not related to the presence or absence of slaughterhouses but is influenced mainly by human activities and behavior towards the definitive host. Dog’s behavior could be a key to understanding this contamination level in areas less likely to develop **E. granulosus** life cycle.

**Canine echinococcosis surveillance in towns of at-risk areas in Uruguay’s control program**

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Cystic echinococcosis caused by the **Echinococcus granulosus** metacestode is endemic in Uruguay. In the surveillance of dogs, it is important to conduct permanent monitoring to identify at-risk areas and assess progress made. In Uruguay, surveillance is carried out in areas identified as at-risk: scattered rural population, small villages and areas of critical socio-economic context in cities. Since 2007 the laboratory of the Zoonosis Commission incorporated the coproELISA diagnostic test (Echinotest) routinely. In the same year the Zoonosis Commission (ZC), with the School of Chemistry, UDELAR, began the elaboration of its own coproELISA (coproELISA-Eg9), with 92% sensitivity and 86% specificity, incorporated in the Program in 2012. Besides rural areas, where work is continuous, an approach was begun to small towns with rural characteristics and areas of critical socio-economic context. From 2008 to 2013, 376 towns distributed throughout the country (18 Departments excluding Montevideo) were studied, with a result in 2008 of 9.9% positive canines, 2% in 2009, 4.6% in 2010, 3.7% in 2011, 6.6% in 2012 and 1.6% in 2013. In 2012, 74 towns and critical socio-economic context areas were studied in 17 Departments. The number of dogs studied was 458. 32 dogs with parasites were diagnosed. Of these, 5 dogs were up to 2 years old (22.7%), 13 were 2 to 8 years old (59%) and 4 (18.18%) were 8 years old or older. 10 had no age data. In 2013, 181 towns were studied in 17 Departments, with 3.238 dogs, 53 of which were positive. The age range was: 14 dogs of up to two years old, 23 from 2 to 8 years, 5 older than 8 and 11 with no age data. In all the cases where infested dogs were identified, the ZC protocol was applied: an epidemiological profile of the house is made and the positive dogs and other dogs in the house are treated, plus an epidemiologic research of the area and environmental anamnesis of the town. The laboratory of the Zoonosis Commission is currently implementing the PCR technique in order to increase the specificity of diagnosis. The risk approach is a fundamental instrument to implement strategies in an efficient and timely manner. Diagnosis in canines is one of the tools that comprise the set of indicators allowing the definition of an at-risk area, added to other parameters such as parasitism of intermediary hosts, environmental contamination, attitudes and practice surveys and analysis of socio-economic and productive conditions.
**Review of cases with alveolar echinococcosis in a tertiary referral hospital in Latvia**

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**Introduction**: The term echinococcosis includes two different diseases – cystic echinococcosis caused by *Echinococcus granulosus* and alveolar echinococcosis caused by *Echinococcus multilocularis*. Although both infectious agents are similar, course of disease is different both clinically and in terms of prognosis.

**Material and methods**: Medical records of a total of 95 patients were used, of which 14 patients with a fully verified diagnosis were selected for analysis. The following information was collected from the clinical records: demographic data (age, sex), diagnostic method (ultrasound (US) examination and parasitological serology), clinical data (symptoms), cyst details (number, localization, and size) and biochemical data of liver markers.

**Results**: Out of 14 selected patients, 11 were female and 3 were male. Patients’ age varied from 13 to 63. Time until diagnosis ranged from the absence of complaints to a period of 180 months; the median time was 6 ±6 months. The most common complaints were pain or discomfort in the abdomen. The main method of examination was ultrasound. The parasitic lesion was localized in the right hepatic lobe (n=8); it was solitary (n=8).

Two of these patients had lesions elsewhere – lungs and kidneys. In about 6 patients there were indications of infiltration of parasitic tissue in the nearby structures, bile ducts and major blood vessels. Analyzing biochemical markers, we found that in 25% of cases they were deviated, median levels of ALT was 50 IU/ml, ASAT - 60.13 IU/ml, AF - 372 IU/ml, GGT – 220.6 IU/ml. Also, only 3 patients had residence in parts of the country where there is higher prevalence of animal cases.

**Conclusions**: Based on data we found it is necessary to create a system that would allow identifying of patients at as early stages of the disease as possible, in order to ensure more efficient treatment and to avoid major impact on patients’ quality of life by the disease.

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**Immunoprophylaxis of secondary alveolar echinococcosis by a protoscolex antigen of Echinococcus multilocularis combined with Ribotan immunostimulation**

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To assess the protective efficiency of immunization against secondary alveolar echinococcosis, with an antigen from *Echinococcus multilocularis* protoscoleces in combination with Ribotan immunostimulation, an assay was carried out on 48 white outbred mice. These were divided into four groups of twelve mice each.

The antigen was isolated from the metabolism products of *E. multilocularis* protoscoleces, cultured in RPMI-1640 medium enriched with 6% fetal bovine serum in a Heraeus CO2 incubator under conditions of high humidity, temperature of 37°C and a CO2 level of 5%.

Ribotan is a new complex immunostimulator consisting of a mixture of low-molecular-weight (0.5-1.0 kD) polypeptides and low-molecular-weight RNA fragments. The mice were immunized via two subcutaneous injections, administered with a ten day interval between them. The formulations consisted of antigen protein (60 µg) and/or Ribotan (5 µl) in 0.2 ml of sterile 0.9% NaCl per injection.

The 1st group of mice was the control, receiving only 0.2 ml of sterile 0.9% NaCl. After a 20 day regimen, the groups were inoculated with a dose of 750 ± 50 units of *E. multilocularis* protoscoleces per mouse.

After 90 days of incubation, the mice were euthanized and dissected for evaluation.

The maximum protection against the *E. multilocularis* infection was obtained in mice immunized with the combination of antigen and Ribotan (92%). Only one mouse in this group showed single metacestodes in the liver, with no infective egg elements. This was proved by a biological test on other mice.

The mice immunized with the protoscolex antigen only showed a protective effect of 58%. In 5 mice from this group, metacestodes with diameters of up to 2mm were found but there were no egg elements.

The protective effect in the group treated with Ribotan only was less than 42%. The majority of mice infected in this group showed fertile parasite metacestodes.

In conclusion, the use of immunostimulators in combination with specific antigens for the immunoprophylaxis against secondary alveolar echinococcosis has a synergistic effect on the treatment.
Initial research for the immune mechanism of CD19+CD24hiCD38hi regulatory B cells and its related molecules in Echinococcus multilocularis infection

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Objective: To observe the phenotype changes and expressions of CD19+CD24hiCD38hi regulatory B cells and its related molecules in the case group infected with Echinococcus multilocularis and relationship between them and focus of infection, in order to research the relationship between CD19+CD24hiCD38hi regulatory B cells and its related molecules in Echinococcus multilocularis infection and to research the function of CD19+CD24hiCD38hi regulatory B cells to E. multilocularis infection.

Methods: Select 38 cases of AE cases group and 40 healthy persons as control group in the First Affiliated Hospital of Xinjiang Medical University from 2013 to 2014 two years. Flow cytometry was used to analyze the rate of CD19+CD24hiCD38hi regulatory B cells in peripheral blood; we used ELISA to measure the levels of IL-10 and TGF-β in each group. The level of liver pathological was observed by HE staining. The protein expression and location of IL-10, TGF-β were analyzed by immune histochemistry respectively.

Results: 1) Flow cytometry results showed that compared with control, the ratio of CD19+CD24hiCD38hi regulatory B cells in peripheral blood B cells of AE cases group had been significantly higher than control group (p<0.05). 2) ELISA results showed that AE cases compared with control, the serum of IL-10 and TGF-β were significantly increased (p<0.05). 3) HE staining shows that the erosion of livers was getting worse during the infection. At the same time, the obvious inflammatory reaction zone can be seen between liver cells and E. multilocularis cyst walls. 4) Comparing with control, the protein expression and location IL-10 and TGF-β were both significantly increased during the infection (p<0.05).

Conclusion: CD19+CD24hiCD38hi regulatory B cells may be the one reason of leading E. multilocularis infection immune suppress. During E. multilocularis infection, CD19+CD24hiCD38hi regulatory B cells in peripheral blood participate in E. multilocularis infection. In the infection, CD19+CD24hiCD38hi regulatory B cells by secreting IL-10 and TGF-β play inhibit function. Furthermore, the high expressions of CD19+CD24hiCD38hi regulatory B cells related molecules IL-10 and TGF-β are disadvantage of host clearance livers E. multilocularis infection and promote E. multilocularis developing.

Keywords: Echinococcus multilocularis infection; CD19+CD24hiCD38hi regulatory B cells; immune escape mechanism

Clinical value analysis of 18F-FDG PET/CT in hepatic alveolar echinococcosis before and after autologous liver transplantation

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Objective: To evaluate the clinical value of autologous liver transplantation in treating advanced liver alveolar echinococcosis (LAE) with 18-F fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT).

Methods: Eight patients’ lesion biodistributions were recorded with 18F-FDG PET/CT before and after surgery. The lesions’ maximum standardized uptake value (SUVmax) was also measured and compared with the pathological results.

Results: The patients’ overall hepatic peri-lesion SUVmax was 3.57 ± 1.2 and the delayed SUVmax was 4.19 ± 1.70. The diagnostic sensitivity of 18F-FDG PET/CT in LAE was 91.67%, with a specificity of 60.00% and accuracy of 82.35%. The positive predictive value was 84.62% and the negative predictive value was 75.00%. SUVmax values of the survived liver were: after 1 month, 1.23 ± 0.78; after 3 months, 1.15 ± 0.67 and after 6 months, 0.85 ± 0.35. Compared with normal liver values (0.95 ± 0.19), the 1-month SUVmax was significantly different. The SUVmax in 3 patients with high-lividity lesions was 2.05 ± 0.72 and the delayed SUVmax was 3.15 ± 0.83; 3 months after transplantation, SUVmax was 1.85 ± 0.62 and the delayed SUVmax was 2.95 ± 0.79, revealing no significant difference.

Conclusions: 18F-FDG PET/CT is effective for determining the biological boundary of LAE and shows important clinical value in determining the metabolic activities of survived liver after autologous liver transplantation.

Keywords: liver alveolar echinococcosis, autologous liver transplantation, positron emission tomography, deoxyglucose
**CT diagnosis of fat-fluid level in hepatic hydatid cyst – 4 cases report and literature review**

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**Background:** Cystic hydatid of liver is a common parasitic disease in Qinghai pastoral areas, imaging characteristic combined epidemiology are easy to diagnose, but in the clinical we found few cystic hydatid has fat-fluid level, this is a rare CT sign, sometimes leading to misdiagnosis.

**Objective:** To analyze 4 cases having fatty contents cystic hydatid proved by pathology combined with literature, raise awareness of this kind of rare signs.

**Clinical case and method:** 3 cases are female and 1 is male; 4 cases are Tibetan nationality, long-term living in Qinghai province pastoral areas. The clinical symptoms are nausea, vomit, diarrhea and other symptoms. Physical examination is only obvious pain in upper abdomen. Laboratory examination is normal. 16 slices Philips Brillianc CT scanner used for abdomen, scanning conditions is 120 Kv og voltage and current of 280mA using non-ionic contrast medium in enhancement scanning, injection rate is 2 ml/s with 70 ml.

**Results:** All cysts have thin and smooth walls, 2 cases with calcification and 2 cases have no obvious calcification walls, All have fatty contents, a fat-fluid level with a superior component that measured -45-80Hu.

**Conclusion:** Typical cystic hydatid signs include calcification of the cyst wall sac, daughter vesicle, internal septa, floating membranes and other common signs, but few cystic hydatid has fat-fluid level; it is a rare sign. It is important to imaging diagnosis; we should know the signs to reduce the misdiagnosis in future cases.

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**Diagnostic Performance of a Native Antigen 5 ELISA for the Human Cystic Echinococcosis**

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Serological diagnosis and follow up of cystic echinococcosis (CE) still lacks standardization of the target antigen, since commercially available tests are generally based on extract of *Echinococcus granulosus* hydatid cyst fluid (HCF). In a previous work, we described a chromatographic method for the preparation of a highly enriched Antigen 5 (Ag5) fraction from HCF. The high reactivity of patient sera against this preparation prompted us to further evaluate this antigen for CE serodiagnosis on a larger sample cohort.

A total of 327 sera from CE patients with heterogeneous clinical conditions (cyst stage, number, localization, previous treatments) and 253 sera from healthy controls were analyzed by an ELISA based on the Ag5 preparation in two different experimental setups (A and B) and, in parallel, by a RIDASCREEN® Echinococcus IgG (R-biopharm) commercial ELISA routinely used in clinical laboratories. Ag5 ELISA setup B revealed an overall sensitivity (95.3%) significantly higher (p < 0.05) than both Ag5 setup A (88.3%) and RIDASCREEN test (87.7%), while differences in specificity (94.1% for Ag5 setup A, 92.5% for Ag5 setup B, and 98.4% for the commercial assay) were not statistically significant.

The relationship between the Ag5 ELISA results and clinical traits were investigated. Chi squared test and bivariate logistic regression analyses highlighted the influence of the pharmacological treatment, the cyst stage and the cyst number on both Ag5 ELISA setups, but the multiple regression analysis indicated that the concurrent dependence of the three clinical variables was statistically significant only for Ag5 setup B.

In conclusion, the two Ag5 ELISA setups revealed different performances. The overall results, given the diagnostic sensitivity, together with the high reliability of the Ag5 preparation method, make this antigen a promising candidate for the serodiagnosis of CE. Further studies should evaluate the ability of our test to provide useful information on specific CE clinical traits.
Studies on diagnosis and epidemiology of echinococcosis/hydatidosis in domestic animals in Romania

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Echinococcosis/hydatidosis is still an important public health and environmental problem in Romania due to lack of coherent surveillance and a control program. In the actual conditions in Romania, all the suspected samples to be infected with hydatid cysts should be send to the National Reference Laboratory for Echinococcosis/hydatidosis where different diagnostic methods are used until the species level.

Here we are presenting the results from 2013 and 2014. The samples were represented by the organs fragment (liver, lung or kidney) with cyst’s collected from intermediate hosts to be examined for the Echinococcus spp larval stage. All samples were macroscopically and microscopically examined in the Parasitology Laboratory of the Institute for Diagnostic and Animal Health. Part of each cyst was collected and sent for the immunohistochemistry technics to detect the specific acid and neutral mucopolisaccharids, using Alsatian blue and Schiff periodic acid staining technics. Germinal layer and/ or protoscoleces were used for the molecular biology to amplify the Echinococcus granulosus genome.

In 2013, were sent to IDAH 571 samples from different intermediary hosts (cattle, sheep, goat and pigs) and 285 (49.9%) were positive for Echinococcus spp. 266 out of 325 from cattle were positive, 14 out of 125 positive samples from sheep, 4 out of 120 positive samples from pigs and the only one sample from goat received was positive.

In 2014, a total of 308 samples were sent from cattle, sheep and pigs from which 170 (55.19%) were positive. From cattle 167 out of 274 were positive, 2 out of 23 sheep samples were positive and one pig sample out of 11 was positive.

The results showed the variation of number of samples which are send and from here the necessity to implement a multiannual control program for echinococcosis/hydatidosis in Romania and to reduce the human infection risk and to decrease or to eradicate echinococcosis/hydatidosis.

Keywords: echinococcosis/hydatidosis, domestic animals, Romania

On dispensary observation of human hydatidosis – 10 years of clinical experience

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In the last decade more than 250 patients with hydatid disease with different organ localization were diagnosed, treated and followed in the Department of Clinical Parasitology at the University Hospital - Plovdiv. They were treated conservatively, with PAIR, and surgically - in a surgical clinic, according accepted indications, while postoperative treatment was conducted too. To specific chemotherapy with albendazole, pathogenic treatment with immunomodulators - Isoprinosine, Res pivax (in pulmonary hydatid disease) and hepatoprotective drug - Ursofalk (in hepatic localization) was included invariably. Treated patients were followed at least five years as monitoring included clinical examination, hematological and serological tests for hydatid disease, targeted imaging - ultrasound, CT and X-ray respectively. During dispensary monitoring the optimal effect of clinical control occurs that confirms the successful treatment, as well as rare cases of recurrent or residual cysts. Clinical supervision of the patient facilitates organizationally discovering of latent cases of hydatid disease among family members or social collective.

Keywords: clinical parasitology, human hydatidosis, treatment and clinical control
Hepatic echinococcosis – the disease that can affect an entire family

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Introduction: The parasitic infection with Echinococcus granulosus or, more rarely, with Echinococcus multilocularis, is a disease known since ancient times, being mentioned by Eber’s Egyptian papyrus around 1550 BC. Batsch, in 1786, was the one who discovered the etiologic agent of the disease and, in Romania, endemic area, the first cases of hydatid disease in humans have been mentioned by Severeanu, Toma Ionescu and Leonte. The main intraabdominal location of the hydatid cyst is the hepatic one. The transmission modality of the disease leads to the possibility that several members of a family, who came into contact with infected animals, to be affected, patients being not aware of the disease, because the symptomatology is poor and non-specific.

Material and method: In the Surgical Clinic of Colentina Hospital, was admitted, in 2011, the female R.M. patient, aged 38 years, with the diagnosis of giant hepatic hydatid cyst, documented through abdominal ultrasound, abdominal CT and positive ELISA test. She underwent surgery, Lagrot intervention being performed. The postoperative evolution was favorable, except the maintaining of an external biliary drainage in increased quantity, which is why we performed ERCP with endoscopic sphincterotomy. By investigating the patient’s family history, we found out that 3 of her 4 children have been diagnosed in another hospital with hepatic hydatid cyst in 2007, two of them undergoing surgery and one receiving only medication. All patients received pre- and post-operative medical treatment with Albendazole. The fourth child and the husband were also investigated, through imaging and serological tests, ascertaining the absence of disease.

Results: Both the patient admitted in Colentina Hospital and her children had favorable evolution after the specific treatment (surgical plus drugs or drugs only), fact that was also attested by the last complete evaluation in 2012.

Conclusions: The presence of Echinococcus infection in one of the family members raises the suspicion that another member of that family may be affected too, justifying a thorough medical anamnesis and a careful imagistic and serological evaluation in order to identify all the possible cases.

Keywords: hepatic echinococcosis, family character

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Opportunities to improve the Department of Clinical Parasitology (Colentina Clinical Hospital) through European programmes

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Aim: The absence of a high quality and equipped laboratory for medical parasitology in Colentina Clinical Hospital from Bucharest made the diagnostic process in humans difficult. This lack has an impact on improving early diagnosis and follow-up of patients. Moreover, national research project could not be carried out at the desired level, due to the technology gap.

Material and Methods: Recently, Colentina Clinical Hospital has been the target for two European programmes, CDPC and HERACLES, which aimed was to bring significant improvements to the healthcare and research practice provided in this center. Colentina – Research Pavilion Development (CDPC), a research infrastructure project, was funded through Structural Funds - Sectoral Operational Programme “Increase of Economic Competitiveness” (2009-2011). Human cystic Echinococcosis ReseArch in Central and Eastern Societies (HERACLES) collaborative project received funds from the Seventh Framework Programme of the European Commission (FP7-HEALTH-INNOVATION-2013).

Results: The Department of Clinical Parasitology provides both traditional and modern diagnostic approaches for parasitic infections, fulfilling European principles of high quality medicine. From the direct examination of different specimens to molecular biology assays, the laboratory is fully equipped, promoting and improving healthcare. Based on these standards, a grant was won by a team from the hospital (HERACLES project), enhancing furthermore the activities in which the laboratory is engaged. Being transformed into a center of excellence, this pavilion of Colentina Hospital is now eligible for many other research studies that can be conducted there, having access to new and efficient techniques. Thus, it can become partner with other research organizations worldwide and achieve great scientific goals.

Conclusions: European projects are strongly associated with raising the standards within the unity where they are implemented. The impact on the health system is reflected both upon patient care and research activities, for a future-oriented medical practice, which demonstrates the importance of these programs and their beneficial outcomes. Recent improvements in the Department of Clinical Parasitology led to the acquiring of a modern laboratory for Colentina Clinical Hospital. Further enquiries into the differences of healthcare practice before and after receiving an external funding should be performed, but the visible development stands for a positive preliminary assessment.

Keywords: European projects, HERACLES, CDPC, parasitology

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Preliminary Results regarding Risk Factors for Cystic Echinococcosis among HERACLES’ US Screened Population in Romania, 2014

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Aims: Cystic echinococcosis (CE) is a parasitic zoonotic disease which is often expensive and difficult to treat. One of the important key factors for disease prevention is public health education. In Romania 45.2% of the population lives in rural areas and have contact with domestic animals especially dogs, sheep, swine. Taking into account the path of transmission of CE we assess the knowledge of rural population on CE risk factors for further action regarding preventive measures to control this disease.

Material and Methods: A descriptive analysis of data gathered from Heracles survey (analyzing the questionnaires filled after signing informed consent) obtained after abdominal US screening for CE and education level related to the risk factors was performed. Data based included 2,902 screened people, in 2014, from the counties of Braila and Giurgiu. Risk factors analyzed refers to contact with animals, slaughterhouse hygiene and antiparasitic drugs prevention.

Results: According to the results for CE, the population screened was grouped in positive - 65 (2.2%) and negative - 2,837 (97.8%). Over 90% of each population group had contact with pigs, sheep and dogs. The secondary level of education (age 14-18) was declared by 1,301 (45.7%) of negative and by 35 (67.3%) of positive screened population. Over 30% of each population groups with secondary studies have the same behavior to dogs: gave uncooked viscera and did not use antiparasitic treatment. Over 80% of both population groups with secondary education level did not dispose viscera of animals by burying or burning. For the primary education level declared by 964 (33.8%) of negative and by 8 (15.4%) of positive screened population, the percentages of risk factors in both groups were: 30% gave uncooked viscera, over 50% did not use antiparasitic dogs treatment and over 90% did not dispose viscera of animals by burying or burning. For people with university level of education the percentage of risk factors follows the same pattern but around 80% of population used antiparasitic treatment.

Conclusions: The percentage of screened population resulted positive to CE was lower and a comparison of CE risk factors among population groups was not possible at this time. The percentage of people using antiparasitic treatment for dogs increased with the education level. Correct hygiene procedures at slaughterhouse level were not respected by population from all educational levels. Further population awareness about CE risk factors and preventive methods are needed.

Acknowledgements: Work funded by FP7 EU Project – HERACLES: grant agreement 602051

Keywords: cystic echinococcosis, risk factors, rural areas

Surgical management of the multiple intraabdominal hydatid cysts

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Background: The intraabdominal extrahepatic placement of the hydatid cysts may be either initial or due of a break / outflow of a hepatic hydatid cyst, especially if there is a large cyst or there are multiple cysts. The lack of suggestive symptoms until the abdominal cysts have grown and have a mass effect, when the patient starts to complain, lead to the delayed presentation of these patients at hospital level.

Material and Method: We present a study achieved in “Colentina” Surgical Clinic which includes 32 patients with extrahepatic intraabdominal hydatid cysts, who underwent surgical procedures, resection and/or conservative, from 01.2005 to 07.2015.

Results: The early postoperative results and the evolutive outcomes were favorable. No relapsed hydatid cysts were found during the follow-up (2-4 years).

Discussions: We must highlight the value and the major role of the medical treatment with albendazole, which works on the larvae of Echinococcus granulosus.

The radical curative treatment is the surgical one. Surgery has the following goals: to evacuate any living cyst found within the abdominal cavity, avoiding if it is possible large tissue resections, to preserve the adjacent organs, to perform procedures which could shrink the risk for relapsed cystic disease. We must underline the importance of PAIR / MoCAT techniques which could be integrated into the surgical approach, in order to minimize the organs’ damage.

Conclusions: The surgical management of those cysts should be accomplished by well-trained surgeons, who could perform various abdominal procedures. Although the hydatid disease may be considered a benign one, the complications could become very dangerous.

Keywords: multiple intraabdominal hydatid cysts, resection procedures, conservative procedures, mini invasive techniques

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**Multiorganic involvement of CE – case study**

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**Aim:** The larval stage of *Echinococcus granulosus*, dogs’ parasite, is responsible for human cystic echinococcosis (CE). Romania is hyper endemic territory for CE. Without an official compulsory notification system, the real epidemiological parameters (incidence, morbidity, mortality) of the disease remain unknown. About 50% of cases are accidentally discovered, but the rest are discovered when complications occur (pruritus, anaphylactic shock, rupture, infection etc). Due to limited financial resources, diagnosis is often delayed. We present a clinical case with multifisural involvement, solved by a multidisciplinary team of specialists.

**Material and methods:** a 42 y.o. man, from Braila, hyper-endemic area (proved by US screening – Heracles Project), with history of type 2 diabetes, presented with persistent dry cough, hemoptysis, dyspnea, fatigue to his GP and later to pneumology. Investigations started.

**Results:** Chest X-ray showed right lung, macronodule-like opacity 5.8/4.8cm, irregular, heterodens and heterocapture; suspicion of lung tumor arose and patient was referred to thoracic surgery. Thorax and abdomen CT was performed; when lung tumor suspected: T2-T3N2 M1. Also, liver, kidney and spleen cysts were found, considered as policystic disease. Thoracic surgery discovered a partially evacuated lung CE, confirmed by pathology. Hematology: no hypereosinophilia found. Biochemistry: normal values. Consequently, patient was referred to parasitology department: serology for CE positive by ELISA and WB. Liver cyst was CE 14.5/4.3cm, CE2 spleen cyst 5.8/2.8cm and CE3a kidney cyst 16/18cm. Albendazole treatment was initiated and kidney cyst was operated in January 2014 (partial cystectomy with drainage). Cyst material showed viable protoscolices and hooks. Movie of the surgical intervention will be presented. One year follow up showed a very good evolution of liver and spleen cysts; no relapses of operated lung and kidney cysts.

**Conclusion:** Hydatid disease can be misdiagnosed even in endemic areas. Specialized multidisciplinary teams and centers for diagnosis and monitoring of the disease are strongly recommended. Surveillance and control programs for the control of the disease should be implemented in Romania.

Work partially funded by Heracles - FP7 EU Project, Grant agreement 602051/2013-2017

**Keywords:** cystic echinococcosis, multi organ involvement, lung CE, kidney CE

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**Modulation of low molecular weight anticoagulant in the surgical treatment of abdominal hydatid cyst**

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**Introduction:** hydatid disease is a widespread illness, both in the world and in Romania, in areas where animal husbandry is practiced. In most cases, surgery is the main pawn in the management of this disease, being completed by the drug treatment. Surgery, regardless the chosen technique, must be accompanied by a properly therapy with low molecular weight heparins, in order to prevent the postoperative complications caused by thrombosis. This treatment can be used in prophylactic dose, as well as in therapeutic dose, in patients which are already having a treatment with oral anticoagulants and / or anti-platelet drugs or which have high thrombotic risk. The presence of a certain degree of hepatic failure, as a result of a complicated hydatid cyst, determines the proper modulation of the anticoagulant therapy.

**Material and method:** in the General Surgery Clinic of Colentina Hospital, there were hospitalized, between 2010 and 2014, 146 patients diagnosed with abdominal hydatid cyst . Among these, 132 underwent open or laparoscopic surgery. In 104 of the patients, who weren’t under oral anticoagulation and / or antiplatelet drugs, nor presented a high thrombotic risk, prophylactic anticoagulation with low molecular weight heparin was established, in the evening before surgery and postoperatively, until active mobilization could be possible. In 28 of the patients, which were under oral anticoagulation and / or antiplatelet therapy at the time of surgery or who had high thrombotic risk, we administered low molecular weight heparins 5-7 days both preoperatively, combined with the arrest of oral anticoagulants, and after surgery, until we could reintroducer the oral anticoagulant therapy.

**Results:** except for 3 cases with superficial venous thrombosis and 2 cases with mild forms of profound venous thrombosis, in all patients the localisation being in the lower limbs, the postoperative course was not marked by the appearance of thrombotic complications.

**Conclusions:** the large scale usage of low molecular weight heparins, both prophylactically and, especially, with curative intent in patients with intraabdominal hydatid cyst undergoing surgery, has the benefit of avoiding the postoperative thrombotic complications, that, in some cases, may develop fatal evolution.

**Keywords:** hydatid disease, anticoagulant therapy, low molecular weight heparins
Preliminary assessment of six recombinant antigens for the diagnosis and follow-up of cystic echinococcosis patients

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Aims: The diagnosis of human cystic echinococcosis (CE) is mainly based on imaging techniques, but reliability of serological confirmation is low due to the lack of standardization and the use of low specific and sensitive crude parasite antigens.

In the HERACLES project (http://www.Heracles-fp7.eu/) one of the objectives is to validate new molecular-based PoC-LoC (Point of Care–Lab on a Chip) kits for serological diagnosis and follow-up of CE. For this reason, cloning, obtaining and purification of 6 recombinant antigens was planned.

Materials and methods: Obtainment of the GST-tagged AFFP, Ag5t, MDH, CaBP, B1t and 2B2t recombinant antigens have been performed at large scale, growing Escherichia coli containing expression vectors for each fusion protein in a fermentor. The proteins were purified on a FPLC (Apta pure). A standardized IgG ELISA protocol was used to test the recombinants and the HF against 601 well characterized sera from 251 CE patients on follow-up, 92 healthy donors and 42 patients with alveolar echinococcosis.

Results: Production of recombinant antigens has been optimized in a simple and affordable process. Three recombinants (Ag5t, B1t and 2B2t) showed an optimal area under the ROC curve (≥0.9) after testing in ELISA. The sensitivity of the six recombinant antigens showed to be influenced by cyst stage (according to WHO-IWGE classification), and number and size of cysts. In general, the reactivity of the recombinant antigens decreased in parallel with the decrease of cyst activity.

Conclusion: Preliminary results in ELISA showed the usefulness of these antigens for the accurate serodiagnosis of CE patients. Furthermore, a characteristic behavior of the antigens was observed for some cyst stages according to the WHO-IWGE classification, helping to understand the evolution of the disease and potentially allowing the follow-up of patients. Next step in the project will be to design PoC (Point of Care) devices containing these CE biomarkers.

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Prediction and identification of antigenic epitopes in Eg95 of Echinococcus granulosus

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Objective: To predict the T and B union epitope of the Eg95 antigen of Echinococcus granulosus, to obtain the different peptide fragment of T-B union epitope via Phage display technology, identify and screen the peptide fragment via Western Blot and ELISA, provide the basis for the T and B union epitope vaccine’s manufacture of Echinococcosis.

Methods: Predicting the B cell and T cell epitope of Eg95 antigen. To analyze the secondary structure, transmembrane structure, B cell epitope, T cell epitope of humans and mice. Finally, determine the area of B cell and T cell union epitope. Using DNAman software design primers, amplified amino acid sequence of T-B cell union epitope, then restructure the gene and the successfully expressed and constructed peptide antibody, confirmed the correctness of epitope peptide antigen by Western Blot. Finally, compared with epitope peptide antigen’s reactivity by ELISA.

Results: Predicted the advantage Eg95 antigen epitope by line through using the bioinformatics method and confirmed 3 T-B union epitope. To successfully cloned and constructed the prokaryotic expression plasmid M13KE/Eg95-1, M13KE/Eg95-2 and M13KE/Eg95-3 plasmid then transformed into E. coli ER2738 respectively, identified the correct sequence using PCR. Prepared the rEg95 patient serum and antiserum as antibody, confirmed the correctness of epitope peptide antigen by Western Blot. Finally, compared with epitope peptide antigen’s reactivity by ELISA.

Conclusion: Predicted the advantage Eg95 antigen T-B union epitope by line through using the bioinformatics method and the successfully expressed and constructed peptide fragment which including the T cell and B cell union epitope through the phage display systems. Using bioinformatics method had an effective means to predict antigen epitope. Constructed 3 epitope in this study have effective antigenicity, which Eg95-2 and Eg95-3 can be potential epitope and development of dominant epitope vaccine.

Keyword: Echinococcus granulosus; Eg95 antigen; bioinformatics; antigen epitope; Phage display
Usefulness and limits of serology for the diagnosis of cystic echinococcosis

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Keywords: cystic echinococcosis, diagnosis, serology

Aim: Hydatid disease (HD) is caused by the larval stages of a tiny (7-8mm length) tapeworm of the genus Echinococcus spp. E. granulosus is responsible for cystic echinococcosis (CE), the most common and widespread form. Persons with CE often remain asymptomatic for years, until the cysts (parasite’s larval stage) becomes large enough to determine discomfort, pain, nausea and vomiting, cough, hemoptysis, etc., according to the location, size, viability. Liver and lungs are the most common locations, but the spleen, kidneys, heart, bone, central nervous system and eyes can also be involved. Diagnosis is based on imagery and serology.

Material and Methods: A retrospective study on 916 serum samples collected from patients admitted in Eco-Para-Diagnostic Medical Center from Bucharest, Romania between 10.01.2013-31.07.2015, samples tested for Echinococcus antibodies using two serological methods (ELISA and Western Blot - WB). From 27 of these patients, biological samples (cysts membranes or hydatid fluid) were collected during surgery.

Results: Out of 910 samples, 491 used ELISA and 419 WB commercial kits. Results of ELISA test were: 88 (17.92%) positives, 37 (7.53%) borderline and 366 (74.55%) negatives. WB performed in parallel, on 419 samples showed: 94 (22.43%) positive, 2 (0.47%) weak positive, 323 (77.10%) negative. Taking into account the positive samples for WB (94) – 43(45.75%) were ELISA positives, 19 (20.22%) negative, 6 (6.38%) borderline and 26 (27.65%) not tested for ELISA.

Out of the 27 biological samples tested for the viability of protoscoleces 25 show the presence of the viable protoscoleces and 2 did not. The samples were collected under surgery from 19 females and 8 males, aged between 5 and 74 years old. Twenty-one out 27 have been also serological tested (ELISA and WB): 13/21 patients-ELISA (6 positive, 7 negative), 8/21 patients WB (5 positive, 3 negative). 2 positive samples had ELISA negative and WB positive, 3 samples had both serological tests (ELISA and WB) negative, 3 were ELISA negative. All the suspect cases showed an image (X-Ray, ultrasound, CT, MRI) evocating CE.

Conclusion: These results show that Western Blot method together with a laboratory examination of the cysts materials increase considerably the accuracy of the diagnosis of CE with negative ELISA. Further studies are necessary to be done in order to evaluate which method (ELISA / Western blot) is more sensitive.

Biliary surgical procedures associated in surgical treatment of hepatic hydatid cyst

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Background: This study analyses biliary surgical interventions that are commonly practiced during hepatic hydatid cyst (HHC) surgery. Biliary surgery can be performed simultaneously or subsequently to HHC resolving procedures. Surgical indications for biliary procedures vary according to the presence and degree of biliary involvement. Giventhat, about 90% of HHCs communicate with bile ducts.

Materials and methods: In the Surgery Clinic of Colentina Hospital, Bucharest between 2010 and 2014, 136 patients with HHC were hospitalized and underwent surgical treatment. Among these, 124 patients had strictly intrahepatic localization, 12 had mixed intra- and extrahepatic localization.

Results: Clinical presentation was that of HHC, in most patients; special biliary presentation features were rare: lithiasic cholecystitis in 7 patients; 3 with acute non-lithiasic cholecystitis; 3 with jaundice, of which 1 associated hydatic cholangitis. Biliary surgery was not required in 47 patients, 4 of whom had previous cholecystectomy. The other 89 patients were cholecystectomised along with main HHC surgical procedures. In 3 cases cholecystectomy was associated with transcystic intraoperative cholangiography. In 8 patients cholecotomy, extraction of cholecystic hydatid and lateral Kehr-type drainage of cholecocutus were performed additionally to cholecystectomy. Postoperative, 41 ERCPs with endoscopic sphincterotomies (ES) were performed in patients with HHC treated by conservative procedures, presenting high flow rates of biliary drainage in a range of 6–35 days (mean 18 days) from surgery date; 6 of these ES were performed in cases with Kehr-type drainage at 9-23 (mean 16 days). Other 4 additional ES were performed in patients operated for HHC in other hospitals.

Conclusions: The majority of biliary interventions associated with HHC surgery were cholecystectomies. Indication for cholecystectomy in HHC patients was individualised and the majority option was for principle cholecystectomy. Cholecocutomies with Kehr-type drainage of cholecystus were necessary in cases with cholangitis or cholecodal hydatid; common bile duct (CBD) surgery imposed cholecystectomy. SE reduced the flow rate and duration of postoperative external biliary drainage of residual cavity, respectively of CBD drainage.

Keywords: hepatic hydatid cyst, cholecystectomy, biliary drainage, endoscopic sphincterotomy

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