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**И.К. АХУНБАЕВ АТЫНДАГЫ КЫРГЫЗ  
МАМЛЕКЕТТИК МЕДИЦИНАЛЫК АКАДЕМИЯСЫНЫН**

# **ЖАРЧЫСЫ**



## **ВЕСТНИК**

**КЫРГЫЗСКОЙ ГОСУДАРСТВЕННОЙ МЕДИЦИНСКОЙ АКАДЕМИИ  
ИМ. И.К. АХУНБАЕВА**

**XXIX ВСЕМИРНЫЙ КОНГРЕСС ПО ЭХИНОКОККОЗУ  
28 - 30 АВГУСТА 2023 г.**

**СБОРНИК МАТЕРИАЛОВ**





**КЫРГЫЗСКАЯ ГОСУДАРСТВЕННАЯ  
МЕДИЦИНСКАЯ АКАДЕМИЯ  
им. И.К. АХУНБАЕВА**

# **Сборник материалов**

**«XXIX ВСЕМИРНОГО КОНГРЕССА  
ПО ЭХИНОКОККОЗУ»**

**28 – 30 августа 2023 г.**

# **Collection of abstracts**

**«XXIX WORLD CONGRESS OF  
ECHINOCOCCOSIS»**

**August 28 - 30, 2023**

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## **Уважаемые участники XXIX Всемирного Конгресса по эхинококкозу!**

Позвольте поблагодарить Вас за то время, которое вы выделили для участия в данном конгрессе, проходящим в Кыргызстане. Кыргызстан добился права проведения XXIX Всемирного конгресса по эхинококкозу впервые на территории Центральной Азии и стран СНГ и объединил более 200 специалистов и экспертов из 23 стран мира.

Конгресс носит важное послание для всего человечества. Эхинококкоз – это заболевание, которое находится в центре внимания медицинской науки и общественного здравоохранения. Распространенность эхинококкоза выходит за границы отдельных регионов и приобретает глобальный характер, охватывая такие территории, как восточная Европа, южная и северная Америка, Африка и, конечно, Центральная Азия.

Важно отметить, что Кыргызстан, как и многие другие страны, не стал исключением. Мы также сталкиваемся с этой серьезной проблемой, и понимаем, насколько важно обменяться знаниями и опытом, чтобы противостоять этому вызову.

Заболеваемость эхинококкозом и альвеококкозом в Кыргызской Республике остается на высоком уровне. Важно отметить, что воздействие эхинококкоза не только ограничивается нашими физическими состояниями, но и имеет социальные и экономические последствия. В Кыргызстане борьба с данным паразитарным заболеванием ведется с 40-х годов прошлого столетия. Кыргызстанские научные школы эхинококкоза всемирно известны и внесли большой вклад в профилактику и лечения данной патологии (школы академика И.К.Ахунбаева, академика К.Р.Рыскуловой).

Исследователи и ученые играют решающую роль в поиске новых подходов к лечению и профилактике эхинококкоза. Врачи, занимающиеся практической медициной, вносят огромный вклад в диагностику и лечение пациентов, страдающих от этого заболевания. Биологи, эпидемиологи, изучая природу и особенности паразита, помогают нам понять, каким образом он взаимодействует с человеческим организмом, что является ключом к разработке эффективных методов борьбы.

Уверен, что опыт, полученный учеными на Бишкекской научной площадке позволит разработать Государственную программу по профилактике и лечению эхинококкоза и альвеококкоза, что даст возможность помочь большему числу больных.

В практической медицине большое внимание следует уделять профилактике или снижению риска возникновения и распространения заболевания, либо остановке его течения, предотвращению передачи, а также усилению профилактических мер и предупреждению заболевания.

Подытоживая свое выступление, хочу отметить, что я горжусь тем, что в нашей стране проходит столь значимое международное мероприятие, которое безусловно даст большой толчок развитию медицинской науки, как в Кыргызстане, так и по всей Центральной Азии.

Надеюсь, вместе мы можем добиться больших успехов в борьбе с эхинококкозом и сделать мир более безопасным и здоровым для всех. Спасибо вам за ваше участие и вклад в это важное дело.



## **Dear participants of the XXIX World Congress on Echinococcosis!**

I am very grateful to you for the time you have allocated to participate in this congress held in Kyrgyzstan. Kyrgyzstan has achieved the right to host the XXIX World Congress on Echinococcosis for the first time in Central Asia and CIS countries and united more than 200 specialists and experts from 23 countries of the world..

The Congress carries an important message for all humanity. Echinococcosis is a disease that is at the center of medical science and public health. The prevalence of echinococcosis goes beyond the borders of individual regions and becomes global, covering areas such as Eastern Europe, South and North America, Africa and, of course, Central Asia.

It is important to note that Kyrgyzstan, like many other countries, has not become an exception. We are also facing this serious problem, and we realize how important it is to share knowledge and experience to face this challenge.

The incidence of echinococcosis and alveococcosis in the Kyrgyz Republic remains high. It is important to note that the impact of echinococcosis is not only limited to our physical conditions, but also has social and economic consequences. In Kyrgyzstan, the fight against this parasitic disease has been underway since the 1940s. Kyrgyz scientific schools of echinococcosis are world famous and have made a great contribution to the prevention and treatment of this pathology (schools of Academician I.K.Akhunbaev, Academician K.R.Ryskulova).

Researchers and scientists play a crucial role in the search for new approaches to the treatment and prevention of echinococcosis. Doctors engaged in practical medicine make a huge contribution to the diagnosis and treatment of patients suffering from this disease. Biologists, epidemiologists, studying the nature and characteristics of the parasite, help us to understand how it interacts with the human body, which is the key to developing effective methods of control.

I am sure that the experience gained by scientists at the Bishkek scientific site will allow us to develop a state program for prevention and treatment of echinococcosis and alveococcosis, which will make it possible to help more patients.

In practicing medicine, great attention should be paid to preventing or reducing the risk of the occurrence and spread of the disease, or stopping the course of the disease, preventing transmission, as well as strengthening preventive measures and prevention of the disease.

Summarizing my speech, I would like to note that I am proud that our country is hosting such a significant international event, which will certainly give a great impetus to the development of medical science both in Kyrgyzstan and throughout Central Asia.

I hope that together we can achieve great success in the fight against echinococcosis and make the world safer and healthier for all. Thank you for your participation and contribution to this important cause.



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## LESSONS LEARNED FROM “PERITAS” INTERNATIONAL PROJECT ON CYSTIC ECHINOCOCCOSIS IN SOUTH AMERICA

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PERITAS (Molecular epidemiological studies on pathways of transmission and long lasting capacity building to prevent cystic echinococcosis infection; <https://www.iss.it/web/iss-en/who-cc-peritas>) is an international collaborative project, aiming to elucidate the pathways of transmission of *Echinococcus granulosus* (Eg), which are poorly understood and have never been systematically investigated [1].

PERITAS was designed as a two-stage project conducted in selected areas of Argentina, Chile and Peru. Stage-1 was a cross-sectional ultrasound-based prevalence study to identify highly endemic clusters of human abdominal cystic echinococcosis (CE) with active cyst stages, where the Stage-2 village-based case-control study was implemented. Stage-2 involved sampling of environmental matrices for molecular identification of Eg in the: a) households/backyards of human positive cases (owners with active CE stages on Stage-1; positive control), b) households/backyards of human negative cases (owners that were CE negative on Stage-1; negative control), and c) village public areas (outgroup) such as squares and parks.

A total of 4,515 people were screened in the regions of Coquimbo (Chile; N=2,439), Rio Negro (Argentina; N=892) and Junin (Peru; N=1,184) [2,3]. The mean prevalence of abdominal CE in these three areas was 1.6%, 4.7% and 3.7%, respectively. Despite COVID-19 pandemic, sampling of matrices were successfully conducted in Chile and Peru in 2020-2021. The percentage of contaminated samples by Eg DNA (both soil samples and dog faeces) was lowest in negative control group, while outgroup common areas had the highest contamination, followed or paralleled by positive control group.

Results suggest that CE should not be solely considered as a “food-borne” disease but an “environmental-borne” disease (including food, water and hand-to-mouth transmissions). Moreover, results stress the shift from the “individual risk” to the “community risk” for acquiring CE in endemic areas.



Figure 1. Map reporting the countries (red) and the centres (logos) participating to PERITAS project. See the website for more details.

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## TRANSMISSION OF ECHINOCOCCUS GRANULOSUS SENSU LATO GENOTYPE 7 IN CAPE VERDE

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**Relevance.** Cystic echinococcosis (CE) is distributed worldwide and is widespread in Africa, where several genotypes have already been identified. However, data on prevalence, distribution and genetic diversity is broadly lacking in most of the African countries [1]. Likewise, to the best of our knowledge, there was no scientific data available on the occurrence of *Echinococcus granulosus*

*sensu lato* (*s.l.*) cluster in Cape Verde. The aim of this study therefore was to investigate the possible occurrence and transmission of *E. granulosus s.l.* in the different islands between domestic dogs and a variety of potential domestic herbivorous mammals [2].

**Materials and Methods.** In this pilot study, environmental dog faecal samples (n=369) were collected around food markets, official slaughterhouses, as well as home and small business slaughter spots in eight of the nine inhabited islands from the Cape Verde archipelago, between June 2021 and March 2022. Additionally, during the same period, 40 cysts and tissue lesions were opportunistically collected from five islands, from locally slaughtered cattle (n=7), goats (n=2), sheep (n=1) and pigs (n=26).

**Results.** Genetic characterisation by a multiplex polymerase chain reaction (PCR) assay targeting the 12S rRNA gene confirmed the presence of *E. granulosus s.l.* in faecal and tissue material. In total, 17 cyst samples from Santiago (n=9), Sal (n=7) and São Vicente (n=1) and eight positive dog faecal samples from Santiago (n=4) and Sal (n=4) were identified as *E. granulosus s.l.* G7 by sequence analysis (*nad2*, *nad5*, and *nad1* genes).

**Conclusion.** The present study confirms the occurrence of *E. granulosus s.l.* G7 in pig, cattle and dog in Cape Verde, disclosing that this genotype, with the potential of zoonotic transmission [3], is circulating in the country. The few positive samples found in the study together with the lack of reports of human cases appears to indicate that CE might not be a serious animal and human health concern in Cape Verde and could realistically be controlled by systematic and coordinated implementation of One Health strategies [4].

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## ДИНАМИКА ЗАБОЛЕВАЕМОСТИ ЭХИНОКОККОЗОМ НА ТЕРРИТОРИИ АЛТАЙСКОГО КРАЯ РОССИЙСКОЙ ФЕДЕРАЦИИ

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**Актуальность.** Алтайский край и республика Алтай традиционно занимают 3-4 место в Российской Федерации по заболеваемости эхинококкозом. До 2020 года мониторинг, статистический учет и анализ заболеваемости проводили на территории Алтайского края по двум нозологиям: эхинококкоз (шифр МКБ X B67.0,1,2,3,4,8,9), альвеококкоз (шифр МКБ X B67.5-B67.7) отдельно. С 2020 года, при сохранении шифров МКБ X пересмотра дифференцированно для эхинококкоза и альвеококкоза, наименование заболевания стало присутствовать в официальной статистике как эхинококкоз, вызванный либо *Echinococcus granulosus* или неуточненный, либо вызванный исключительно *Echinicoccus multilocularis*, при точной верификации диагноза, что вызывает вопросы как к точности диагностики, так и к эпидемиологии форм эхинококка на мониторируемой территории.

По последним литературным данным, эхинококкоз относится к группе наиболее распространенных и наиболее тяжелых по клиническим проявлениям гельминтозов на территории Алтайского края, поэтому не утрачивает своей актуальности для клинической практики. Учитывая тяжесть заболевания, широкий спектр целевых органов для личиночных форм, длительную (до 75 лет!) латентность, тяжелое течение и множественные осложнения заболевания, часто приводящие к калечащим операциям, инвалидизации и даже смерти пациентов [1,2,3], необходим постоянный мониторинг заболеваемости гельминтозом и анализ рисков заражения населения территории края.

**Цель исследования.** Анализ динамики заболеваемости эхинококкозом на территории Алтайского края в первой четверти XXI века, с учетом форм эхинококкоза и типичных целевых органов гельминта в организме человека.

**Материалы и методы исследования.** Статистические данные о ежегодной заболеваемости эхинококкозом в Алтайском крае.

**Результаты.** Анализ заболеваемости эхинококкозом в Алтайском крае выявил следующие особенности: вариативность ежегодной заболеваемости от 0 случаев (ряд лет наблюдений с 2000 до 2023 года), до 18 в 2011 и 16 в 2012 годах. Смертные случаи констатировали в 2003, 2007, 2008, 2009, 2010, 2011, 2012, 2013 гг., по одному в год. Анализ причин смерти показал непосредственную связь с заболеванием гельминтозом, в ряде случаев установление диагноза как причины смерти стало возможным только посмертно. Низкие цифры выявления заболевания эхинококкозом в начале периода наблюдений (2000-2003 годы) можно связать со сложностями диагностики в посткризисный период (1998г.), что может быть объяснено низкой выявляемостью заболевания, обусловленной плохой оснащенностью лабораторно-клинической службы в посткризисные годы. Пик заболеваемости наблюдали в 2011-2012 годах, что также является посткризисным эффектом кризиса 2008-2009 гг., и связано скорее с экономическими причинами изменения структуры питания населения и снижением контроля за стихийными рынками. В 2020 году отмечали нулевой показатель заболеваемости, что объяснимо при органичении плановой медицинской помощи в период пандемии. Флуктуации заболеваемости по региону составили за 23 года наблюдений 8,75 раза. За период наблюдений преимущественно выявляли эхинококкоз, вызываемый *Echinococcus multilocularis* до 95-100% от ежегодной заболеваемости и только единичные находки - *Echinicoccus granulosus* (0-5% от заболеваемости за год). Целевым органом для обеих форм эхинококкоза являлась печень, спорадические случаи 0-2 в год в заболеваемости составлял эхинококкоз легких. За весь период наблюдений практически не было случаев фиксации случаев эхинококкоза центральной нервной системы и органов чувств.

**Заключение.** Учитывая тяжесть заболевания эхинококкозом, его длительную латентность, сложности диагностики, ассоциированность заболеваемости с кризисами в социуме необходим постоянный мониторинг выявляемости заболевания и его рисков как на территории Алтайского края, так и сопредельных регионов, на территории которых формируются общие экосистемы, обеспечивающие поддержание очагов эхинококкоза обеих форм, что имеет безусловно как эпидемиологическое, так и экономическое значение для вовлеченных в очаги эхинококкоза территорий.

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**SYSTEMATIC ANALYSIS FOR THE GLOBAL BURDEN OF DISEASE STUDY 2019:  
THE IMPACT OF HUMAN CYSTIC ECHINOCOCCOSIS  
IN THE CENTRAL ASIAN REGION, 1990–2019**

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This research aims to update knowledge on the regional and national sickness burden attributable to CE from 1990 to 2019, as well as epidemiology and disease control, with a particular emphasis on the People's central Asian regions. In 2019, the three greatest numbers of CE cases were recorded in Kazakhstan (23986; 95% UI: 19796; 28908); Uzbekistan (41079; 18351; 76048); and Tajikistan (10887; 4891; 20170) among all 9 countries. The three countries with the greatest ASIR of CE were estimated to be Kazakhstan (127.56; 95% UI: 105.34-153.8), Uzbekistan (123.53; 95% UI: 58.65-219.16), and Tajikistan (121.88; 58.57-213.93). Kyrgyzstan, Tajikistan, and Uzbekistan had the biggest increases (125%, 97%, and 83%, respectively) in the number of incident cases of CE, whereas Georgia, Kazakhstan, and Armenia saw the largest decreases (45%, 8%, and 3%, respectively). Moreover, the ASIR of CE increased the most in Kyrgyzstan [EAPC = 1.3%, 95% UI: (0.94% to 1.67%)], while it decreased the most in Kazakhstan [EAPC = 1.22%, 95% UI: (1.44% to 0.99%)] and Georgia [EAPC = -1.04%, 95% UI: (1.23% to - 0.85%)]. To reduce the illness burden caused by CE, our findings may help public health professionals and policymakers design cost-benefit initiatives.

**UNVEILING THE HEALTH IMPACT OF HUMAN CYSTIC  
AND ALVEOLAR ECHINOCOCCOSIS IN EUROPE.**

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Cystic (CE) and alveolar (AE) echinococcosis affects mainly pastoral and rural communities in both low-income and upper-middle-income countries. In Europe, CE and AE should be regarded as orphan and rare diseases. Although human CE and AE are notifiable parasitic infectious disease in most European countries, in practice they are largely under-reported by national health systems.

To fill this gap, we extracted data on the number, incidence, and trend of human cases in Europe through a systematic review approach within MEME project [1], using both the scientific and grey literature and accounting for the period of publication from 1997 to 2023. The highest number of possible human cases at the national level was calculated from various data sources to generate a descriptive model of human CE and AE in Europe.

Regarding CE, we identified 64,745 human cases from 40 European countries [2]. The mean annual incidence from 1997 to 2020 throughout Europe was 0.64 cases per 100.000 people. Based on incidence rates and trends detected in this study, the current epicentre of CE in Europe is in the south eastern European countries, whereas historical endemic European Mediterranean countries have recorded a decrease in the number of cases over the time.

Regarding AE, the ongoing research currently identified 3,987 probable or confirmed human cases from 40 European countries. The mean annual incidence, from the index case after 1997 to 2023 throughout Europe, was 0.07 cases per 100.000 people. Switzerland and Lithuania recorded the highest incidences. Based on incidence rates detected in this study, two major epicentres has been identified in Europe: the Alpine area and the Baltic area.

CE and AE in Europe remain a relevant public health issue and findings from this study should be used to support the planning of surveillance and control programmes in Europe according to the WHO 2021-2030 roadmap for neglected tropical diseases [3].

**Acknowledgments.** This research was funded by the MEME project from the EU's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme.

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## **ACHIEVEMENTS OF THE “MEME” INTERNATIONAL PROJECT ON ECHINOCOCCUS SPP. IN EUROPE AND BEYOND**

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MEME (Multi-centre study on *Echinococcus multilocularis* and *Echinococcus granulosus* s.l. in Europe: development and harmonization of diagnostic methods in the food chain) is an international collaborative project funded by the European Union under the Framework of One Health European Joint Programme [1]. MEME aims to fill research gaps highlighted by international agencies for the standardization of detection methods, production of new molecular tools and epidemiological data at large scale on the zoonotic parasites *Echinococcus multilocularis* (Em) and *Echinococcus granulosus sensu lato* (Eg), causing alveolar echinococcosis (AE) and cystic echinococcosis (CE), respectively [2].

MEME achievements were: 1. The production of SOPs for the sampling of different matrices from naturally or experimentally infected definitive and intermediate animal hosts; 2. The validation of the parasitological (SSCT) and molecular diagnostic (multiplex- and MC-RT-PCRs) procedures to detect

Em and Eg in different matrices along the food chain; 3. The development, validation and comparison of new molecular tools (comparison of DNA extraction and PCRs assays, novel probe-based qPCRs, PCR-RFLPs and multiplex PCR assays and NGS approach); 4. Multicentre studies for the production of data relevant for epidemiological assessments: contamination of fresh vegetables for human consumption by Em/Eg; prevalence of Em/Eg in dog faeces; 5. Quantitative assessment on the impact of human CE in Europe by means of systematic review approach; 6. Molecular and clinical epidemiology studies in selected geographical areas [1].

MEME impacted on animal health, public health and food safety sectors. Beneficiaries of scientific outputs of MEME are EU reference labs, international organizations and all decision makers. MEME provided a set of molecular tools, epidemiological risk assessments and quantitative epidemiological models for the detection, surveillance and control of these parasitic infectious diseases in Europe and beyond.

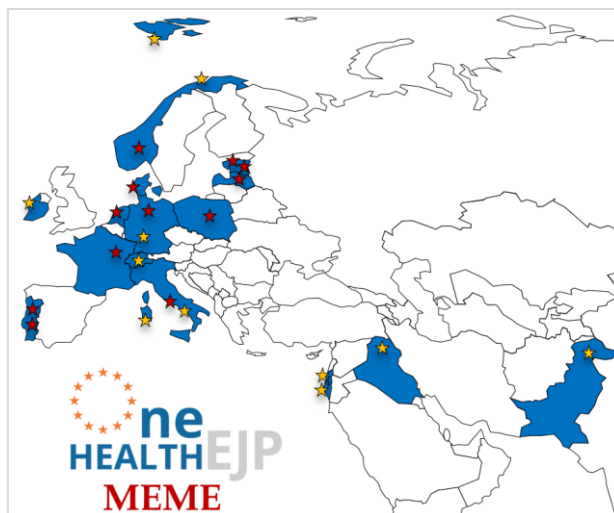


Figure 1. Map reporting the countries (dark blue) and funded (red stars) or external (yellow stars) centres participating to MEME project. See the [website](#) for more details.

**Acknowledgments.** This research was funded by the MEME project from the EU's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme.

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## UNVEILING THE IMPACT OF HUMAN ALVEOLAR ECHINOCOCCOSIS IN EUROPE DURING 1997-2023

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In Europe, alveolar echinococcosis (AE) is mainly transmitted by wildlife in rural communities, where the environment is contaminated by parasitic eggs of *Echinococcus multilocularis*. AE in humans is a chronic disease, often life-threatening since it causes a tumour-like progression. In Europe, AE should be regarded as an orphan and rare disease. Although human AE is a notifiable parasitic infectious disease in most European countries, in practice it is largely under-reported by national health systems.

To fill this gap, we extracted data on the number, incidence, and trend of human cases in Europe through a systematic review approach within MEME project [1], using both the scientific and grey literature and accounting for the period of publication from 1997 to 2023. The highest number of possible human cases at the national level was calculated from various data sources to generate a descriptive model of human AE in Europe.

This is an ongoing research in which 3,911 probable or confirmed human AE cases from 40 investigated European countries have been currently identified during the considered period. Case fatality rate during the period was 7.65%. Austria, France, Germany, Switzerland, Lithuania, Poland, Slovak republic and Switzerland accounted for 91.5% of all AE documented cases. The current mean annual incidence during the period throughout Europe was 0.07 cases per 100.000 people from 24 countries. Based on incidence rates detected in this study, two major epicentres has been identified in Europe: the Alpine area and the Baltic area. AE in Europe remains a relevant public health issue and findings from this study should be used to support the planning of surveillance and control programmes in Europe according to the WHO 2021-2030 roadmap for neglected tropical diseases [2].

### Acknowledgments

This research was funded by the MEME project from the EU's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme.

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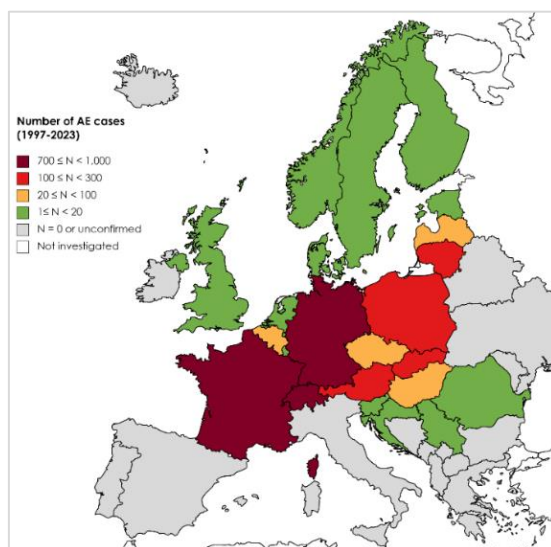


Figure 1. Preliminary map with number of documented human alveolar echinococcosis cases in Europe at the national level during 1997-2023 (n=3,799).



## UNVEILING THE INCIDENCES AND TRENDS OF HUMAN CYSTIC ECHINOCOCCOSIS IN EUROPE DURING 1997-2022

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Cystic echinococcosis (CE) affects mainly pastoral and rural communities in both low-income and upper-middle-income countries. In Europe, it should be regarded as an orphan and rare disease. Although human CE is a notifiable parasitic infectious disease in most European countries, in practice it is largely under-reported by national health systems.

To fill this gap, we extracted data on the number, incidence, and trend of human cases in Europe through a systematic review approach within MEME project [1], using both the scientific and grey literature and accounting for the period of publication from 1997 to 2021. The highest number of possible human cases at the national level was calculated from various data sources to generate a descriptive model of human CE in Europe.

We identified 64,745 human CE cases from 40 European countries [2]. The mean annual incidence from 1997 to 2020 throughout Europe was 0.64 cases per 100.000 people. For the years 2017-19, we identified a total number of cystic echinococcosis cases four-fold higher than for The European Surveillance System (TESSy) data.

Based on incidence rates and trends detected in this study, the current epicentre of cystic echinococcosis in Europe is in the south eastern European countries, whereas historical endemic European Mediterranean countries have recorded a decrease in the number of cases over the time. Cystic echinococcosis in Europe remains a relevant public health issue and findings from this study should be used to support the planning of surveillance and control programmes in Europe according to the WHO 2021-2030 roadmap for neglected tropical diseases [3].

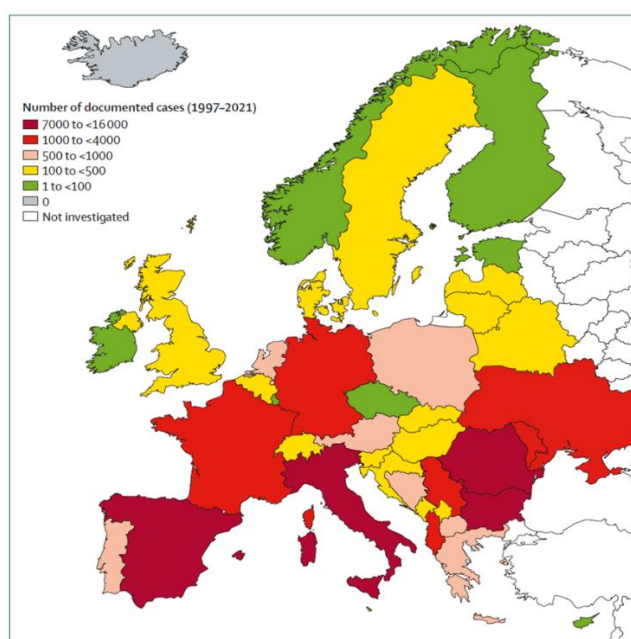


Figure 1. Number of documented human cystic echinococcosis cases in Europe at the national level during 1997-2021 (n=64,745).

**Acknowledgments.** This research was funded by the MEmE project from the EU's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme.

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## **ECHINOCOCCOSIS IN NORTH AMERICA: PAST, PRESENT, AND FUTURE DIRECTIONS**

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A neglected disease globally, it may be underrecognized and emergent in regions of the United States. Prior to molecular tools, historical context in North America included successful control of pastoral forms, impacts to vulnerable communities, and disease introduction from animal movements. Today, despite ongoing sylvatic endemicity and unknown risk to people, this disease complicates wild wolf reintroductions within the US. Differences in pathogenicity and host preferences by cryptic genotypes of *Echinococcus* spp. warrant re-analysis of reservoir hosts and landscape risks to people of North America in a contemporary One Health context. To update our understanding of echinococcosis in US patients from recent decades, we performed retrospective analysis of de-identified diagnosis codes in a federal shared healthcare network database (TriNetX). We found >36,000 diagnoses of human echinococcosis in the US from 2003-2023, with 49 being alveolar echinococcosis and 454 being cystic echinococcosis, the remaining being “unspecified” diagnoses. Demography, spatial distribution, treatment rates and survival differ by reported *Echinococcus* species. Sepsis, pleural fluid abnormalities and increased mortality risk were significant for these patients. The geography of these results is compared to animal infections, with discussion of future opportunity for improved surveillance and management.

## **СРАВНИТЕЛЬНАЯ ЭПИДЕМИОЛОГИЧЕСКАЯ ОЦЕНКА ИНВАЗИИ КИСТОЗНОГО И АЛЬВЕОЛЯРНОГО ЭХИНОКОККОЗА НАСЕЛЕНИЯ АЛАЙСКОГО И КОЧКОРСКОГО РАЙОНОВ КЫРГЫЗСКОЙ РЕСПУБЛИКИ**

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В настоящее время проблема эхинококкоза и альвеококкоза остается актуальной как для Кыргызской Республики (КР), так и для эндемичных по данной нозологии Алайского района Ошской области и Кочкорского района Нарынской области. Важным компонентом эпидемиологического надзора за кистозным (КЭ) и альвеолярным эхинококкозом (АЭ) является инструментальное наблюдение (скрининг) с целью ранней диагностики и определения достоверности карты распространения. В статье представлены результаты инструментального (УЗИ) обследования условно здорового населения 56 сел двух (Алайского, Кочкорского) районов Кыргызской Республики за 2016год. В ходе исследования выявлена высокая распространенность как альвеолярного, так и кистозного эхинококкоза. Для АЭ распространенность составила 7,1% [5,7%-8,9%] в Алае, что значительно выше, чем в Кочкорском районе, где распространенность составила 3,6% [1,8%-3,9%]  $p<0,01$ . Распространенность КЭ были ниже в Алае с распространенностью 0,9% [0,4%-1,6%], тогда как в Кочкоре с распространенностью 1,7% [1,0%-2,7%],  $p<0,01$ .

Только у мужчин АЭ встречалась чаще и то только в центре Кочкора. Полученные в ходе исследования результаты могут свидетельствовать о высоком уровне фактической заболеваемости населения данным гельминтозом в исследуемой популяции и свидетельствовать о необходимости проведения целенаправленных ранних противоэпидемиологических и профилактических мероприятий.

## THE ROLE OF STRAY DOGS ON ALVEOLAR ECHINOCOCCOSIS IN THE NORTHEASTERN TURKEY

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Alveolar echinococcosis (AE) is a zoonotic disease caused by *E. multilocularis*, which has a high mortality if left untreated [1]. The aim of this study was to determine the role of stray dogs through the presence and prevalence of *E. multilocularis* in stray dogs in Northeastern Region, Turkey.

In the seven provinces, 1069 fecal samples were collected in 2019-2020. The fecal samples were analyzed SSFM for isolation of taeniid eggs. Each sample which positive taeniid egg was analyzed by PCR for *E. multilocularis*.

Taeniid-type egg positivity was determined as 26.8% (286/1069). *E. multilocularis* was detected in 8.7% (93/1069) by PCR.

The results of this study reveal the presence and high prevalence of *E. multilocularis* in stray dogs in the provinces of the Northeastern Region. These results also highlighted that dogs have a very important role in the transmission of AE, which is an important public health problem for humans in the region.

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## GLOBAL EPIDEMIOLOGY AND SITUATION OF CYSTIC ECHINOCOCCOSIS IN TURKEY

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Cystic echinococcosis (CE) has been recognized since 1950 as a public health problem by WHO. And echinococcosis is included in the list of 17 neglected tropical diseases (NTD) (WHO, 2013) and in the list of priority neglected zoonotic diseases. So, it is globally distributed and found in every continent except Antarctica. In endemic regions, human incidence rates for CE can reach more than 50 per 100 000 person-years, and prevalence levels as high as 5%–10% may occur in parts of Argentina, Peru, East Africa, Central Asia and China. It is especially present in the Mediterranean and Eastern countries, its cycle mainly involving domestic dogs and ruminants (sheep, goats, cattle etc.). CE is responsible for significant economic losses in the public and animal health sector. At global level, Budke and colleagues (2006), estimated a human burden of around 1 million Disability-Adjusted Life Years (DALYs) and 760 million US\$ losses due to human infection, and annual livestock production losses of at least US\$ 140 million.

Cystic echinococcosis has been recognized in Turkey since 1861 and occurs throughout Turkey, whereas AE predominantly occurs in the Eastern Anatolia region. It has a negative impact on the national economy as well as the health, and still remains one of the most important and serious helminthic diseases. Exposure of humans to *E. granulosus* is common due to the lifestyle of the people who live in close proximity to sheep and dogs. Most people live in rural areas and are engaged in animal husbandry. It is quite common due to the wide prevalence of stray dogs and the lack of necessary sanitary precautions (Altintas, 2003; Šnábel, 2009). Field studies on the epidemiology of CE in our country is limited. Although the present number of stray dogs and owned dogs is unknown in Turkey, according to some of the local studies, *E. granulosus* infection in dogs ranges between 0.32% and 40%. The prevalence of cattle among intermediate host butchery animals (slaughter animals, slaughtered animals, livestock animals) varied between 6,8–34,3% in Eastern Anatolia while was 3,4% in Central Anatolia, 11,3% in the Black Sea and 4,0% in the Marmara Region. The prevalence in sheep; was between 31,7–46,4% in Eastern Anatolia, 4,9% in Central Anatolia, 6,5% in the Black Sea and 22,9% in Marmara Region (Altintas N, et al., 2020). After establishing the Turkish Association of Hydatidology (TAH) in 1999 which has been started to discuss with the authorities from Ministry of Health and Ministry of Agriculture, CE became a “notifiable disease” in 2005. TAH is the only Non Governmental Organization (NGO) in Turkey that works on “cystic echinococcosis”. Recently, Turkey Zoonotic Diseases Action Plan (2019–2023) is prepared by Zoonotic Diseases Sub-Commissions of Turkey Zoonotic Diseases National Committee with participation of Ministry of Agriculture and Forestry and other institutions in coordination of Ministry of Health. And the report is prepared by Cystic Echinococcosis Commission of Turkey Zoonotic Diseases National Committee to determine the current status of the disease in order to increase the efficiency of health services related to the disease for control of CE. Control programme development has been started to discuss. So, CE urgently needs attention both for protecting public health and animal welfare in Turkey.

**Key words:** Cystic echinococcosis, epidemiology, Turkey

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## ФАКТОРЫ РИСКА ЭХИНОКОККОЗА В КЫРГЫЗСКОЙ РЕСПУБЛИКЕ

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**Актуальность.** Эхинококкоз – паразитарная инвазия, вызываемая цестодой рода *Echinococcus*. Кыргызская Республика (КР) – страна в Центральной Азии, которая славится чрезвычайно высокой заболеваемостью эхинококкозом. Эпидемия эхинококкоза в КР началась после распада Советского Союза и была связана с изменением методов ведения сельского хозяйства: закрытие крупных организованных колхозов и прекращение кочевого овцеводства, отделявшего животноводство от земледелия. Пик заболеваемости пришелся на 2014 г. и составил 20,2 в год на 100 000 населения. Основные усилия по контролю заболеваемости предпринимались с 2008 г., и после 2016 г. наблюдалось медленное снижение заболеваемости. Несмотря на эти успехи, заболеваемость остается на высоком уровне [1, 2, 3]. Всего в Ошской, Нарынской и Баткенской областях КР в 2015-2017 годах проведено ультразвуковое обследование и опрошено на наличие потенциальных факторов риска 10093 человека. Распространенность кистозного эхинококкоза (КЭ) колебалась от 0,2 до 25,2% в исследуемых регионах. Типичные факторы, такие как наличие собаки или крупного рогатого скота, слабо влияли на риск КЭ (ОР 1,18–1,83). Использование колодезной воды и наличие кошки в большей степени влияли на риск КЭ (ОР 2,03–2,28). Факторы риска КЭ были сильно разнородны в разных регионах исследования, причем закономерности не всегда соответствовали классическим путям передачи биогельминтозов (отсутствие риска от крупного рогатого скота в определенных районах, высокий риск от использования колодезной воды, выращивания овощей, содержания кошек). Таким образом, рост КЭ в КР не является целостной с точки зрения потенциальных механизмов и факторов риска, и определенные области могут значительно выиграть от профилактических мер, эффективность которых в других местах будет ограниченной.

**Целью исследования** являлась оценка поведенческих факторов риска заражения населения эхинококкозом и альвеококкозом в эндемичных районах Нарынской, Ошской и Баткенской областей КР. По результатам ретроспективного анализа за период 2000-2020 гг. по среднему показателю заболеваемости эхинококкозом в КР первое место среди жителей занимает Нарынская область 17,9% и второе место Ошская область - 14,8%. В связи с этим, в исследование включены две области нашей страны. Баткенская область взята для контроля.

**Объект исследования** – население Ошской, Нарынской и Баткенской областей КР. Место исследования – населенные пункты Ошской, Нарынской и Баткенской областей, 2015-2017 гг. Материалы исследований. В 2015–2017 годах было проведено ультразвуковое обследование и опрошено на наличие потенциальных факторов риска 10093 человека. Методы. Эпидемиологический, статистический метод, социологический опрос населения, анкетирование. Статистическая значимость отношения шансов были рассчитаны с использованием [www.medcalc.org/calc/odds\\_ratio.php](http://www.medcalc.org/calc/odds_ratio.php).<sup>13</sup> Значение р-было рассчитано, как в [4], стр. 542. Для массового опроса населения нами была разработана анкета, содержащая 28 вопросов, при ответах на которые давались сведения о наличии сельскохозяйственных и домашних животных, ветеринарно-санитарном надзоре за ними, санитарно-бытовых условиях проживания и поведенческих факторах риска. Все вопросы были открытыми. В исследовании участвовали жители выше перечисленных областей. У каждого респондента были получены в письменном виде согласие на участие в исследовании. От каждого респондента было получено письменное согласие на участие в исследовании.



**Этическое заявление.** Этическое одобрение исследования было получено комитетом по этике при научно-производственном объединении «Профилактическая медицина» МЗ КР (выписка из протокола №1 от 19.02.2010 г.). Авторы заявляют, что исследование проводилось при отсутствии каких-либо коммерческих или финансовых отношений, которые могли бы быть истолкованы как потенциальный конфликт интересов.

**Результаты.** Распространенность эхинококкоза в исследуемых регионах (Нарынской и Баткенской областях или в районах Ошской области) колебалась от 0,2% (Араванский район) до 25,2% (Ноокатский район). Типичные факторы риска, такие как наличие собаки или овец, слабо влияли на риск эхинококкоза (отношение рисков, ОР 1,2–1,8). Единственным значимым «профессиональным» фактором риска был статус пенсионера (ОР 2,12,  $p < 0,0001$ ), что может быть связано с более высоким кумулятивным риском заражения. При этом статус домохозяйки, связанный в других исследованиях с риском эхинококкоза, в нашем исследовании был даже отрицательным фактором (ОР 0,76; 95% ДИ 0,65–0,93;  $p < 0,05$ ). Факторы риска в исследуемых регионах значительно различались. Владение домашним скотом было значимым фактором риска только в районах Ноокат и Кара-Кулжа, владение собакой – только в районе Ноокат и в Нарынской области. Профиль факторов риска не всегда соответствовал классическим путям передачи эхинококкоза (отсутствие риска от содержания скота и собак во многих районах). При этом выявлен высокий и статистически значимый риск от факторов риска, характерных для геогельминтозов – использование воды из колодца (ОР 2,0; 95% ДИ 1,5–2,8;  $p < 0,0001$ ), выращивание овощей на продажу (ОР 1,4; ДИ 1,1–1,7;  $p < 0,001$ ) и наличие кошек (ОР 2,28; ДИ 1,8–2,8;  $p < 0,0001$ ), которые могли выступать фактором механического переноса яиц в дом. Хотя некоторые из этих факторов теоретически могли быть статистическим артефактом, связанным с образом жизни, нам такие связи (например, владение кошкой и статус пенсионера) выявить не удалось. В некоторых районах отмечены крайне высокие значения факторов риска, например, владение лошадью в районе Кара-Кулжа (ОР 23,2; ДИ 6,4–84,4;  $p < 0,001$ ), что, скорее всего, является статистическим артефактом, но подчеркивает гетерогенность эпидемии по регионам.

**Обсуждения.** КР известен высокой заболеваемостью и распространенностью, как эхинококкоза, так и альвеококкоза. По нашим данным, общая распространенность эхинококкоза в эндемичных регионах составила 4,5%. Это близко к оценке 3,4%, опубликованной почти 20 лет назад [5]. Единственным профессиональным статусом, существенно влияющим на распространенность эхинококкоза, были пенсионеры. Это соответствовало более высокой распространенности эхинококкоза среди возрастной группы 60+. Потенциально это можно объяснить кумулятивным воздействием в течение жизни. Однако такая тенденция кумуляции распространенности в целом наблюдалась не во всех возрастных группах, и доля пострезекционных пациентов с возрастом существенно не увеличивалась. Факторами риска эхинококкоза в Турции были пенсионеры и домохозяйки [6]. В нашем исследовании все другие профессии, включая домохозяйку и фермерство, были отрицательно (хотя и незначительно) связаны с риском КЭ. Охота (включенная в анкету как фактор риска альвеококкоза) оказалась значимым фактором риска эхинококкоза с ОР 2,85. А также в КР хорошо развита скотоводство, у большинство больных эпидемиологические факторы риска инфицирования связаны с наличием большой численности домашних животных [7]. Были убедительные доказательства передачи при прямом контакте с собаками как для КЭ, так и для альвеолярного эхинококкоза (АЭ). Расчетные атрибутивные доли составили 26,1% (ДИ 13,8%–39,6%) и 34,4% (ДИ 20,7%–48,2%) соответственно. По оценкам, передача через загрязненную воду является причиной примерно 29,4% (ДИ 12,1%–51,7%) для КЭ и 24,8% (СИ 10,6% до 42,6%) для АЭ. Загрязненная пища может быть причиной примерно 23,4% случаев КЭ (ДИ 2,1–47,3%) [8]. Исследования, проведенные в 2001–2003 гг. в Польше, показали высокую распространенность солитера у рыжих лисиц на северо-востоке (34,5%) и юго-востоке (39,3%) Польши с очагами инфекции в некоторых уездах (до 70%) заражены лисы). Это обуславливает высокий потенциальный риск заражения людей в этих районах [9]. Оценка потенциальных факторов риска, связанных с кистозным эхинококкозом, показала



высокую значимость следующих факторов: демографические (возраст), социальное положение (уровень образования) и гигиенические практики (удаление собачьих фекалий и использование перчаток). Дети до 18 лет и пожилые люди считаются возрастными группами наибольшего риска в Монголии, социальные условия (уровень образования) и правила гигиены (уборка собачьих фекалий и использование перчаток) [10]. В результате поиска в базе данных было выявлено в общей сложности 1026 исследований, 26 из которых подходили для данного метаанализа. Совокупная распространенность АЭ и КЭ составила 2,88% и 5,66% соответственно. Этническая принадлежность ( $OR = 2,93$ , 95% ДИ: 1,81–4,75;  $I^2 = 0$ ), профессия пастуха ( $OR = 2,66$ , 95% ДИ: 2,25–3,14;  $I^2 = 8,0\%$ ), не мытье рук перед едой ( $OR = 2,40$ , 95% ДИ: 1,34–4,28;  $I^2 = 82,8\%$ ) и принадлежность к женскому полу ( $OR = 1,45$ , 95% ДИ: 1,26–1,66;  $I^2 = 33,9\%$ ) были факторами риска КЭ. В первую пятерку факторов риска КЭ входили этническая принадлежность ( $OR = 3,18$ , 95% ДИ: 1,55–6,52;  $I^2 = 79,2\%$ ), кочевой образ жизни ( $OR = 2,71$ , 95% ДИ: 1,65–4,47;  $I^2 = 55,8\%$ ), питье некипяченой воды ( $OR = 2,47$ , 95% ДИ: 1,36–4,47;  $I^2 = 85,7\%$ ), кормление собак внутренностями ( $OR = 2,35$ , 95% ДИ: 1,89–2,91;  $I^2 = 21,5\%$ ), занятие пастухом ( $OR = 2,19$ , 95% ДИ: 1,67–2,86;  $I^2 = 85,1\%$ ) [11].

**Заключение.** Разный уровень заболеваемости и разный профиль рисков указывают, что эпидемия эхинококкоза в КР не является единой целостной эпидемией с точки зрения потенциальных механизмов и факторов риска. Для успешной борьбы с эхинококкозом необходимо проводить детализированную оценку факторов риска и разработку первоочередных мероприятий как минимум на уровне районов, а, возможно, и более мелких административных образований, поскольку некоторые районы могут значительно выиграть от профилактических мер, эффективность которых в других местах будет ограниченной. Профиль факторов риска указывает, что профилактика должна быть не столько индивидуальной, сколько коммунальной.

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## CYSTIC ECHINOCOCCOSIS IN SUB-SAHARAN AFRICA (CESSARI PROJECT)

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**Relevance.** A considerable amount of knowledge on echinococcosis has been available from the sub-Saharan region, but research has largely concentrated on few foci (e.g. the Turkana region in Kenya). The aim of CESSARi was to fill gaps of knowledge on geographical distribution, frequency and epidemiology of different *Echinococcus* species, identify local scientific expertise, strengthen research facilities and encourage communication between African institutions.

**Materials and methods.** From 2009 to 2019, a network was funded by the German Research Foundation (DFG), that included researchers of thirteen medical, veterinary and biological research institutions in Sudan, Ethiopia, Kenya, Uganda, Ghana, Zambia, Namibia, South Africa and Germany as key partners, plus several associated researchers from elsewhere. Five basic molecular laboratories were established or upgraded, and four mobile ultrasound units were provided. International training courses were organised for molecular biology, ultrasound diagnostics, percutaneous intervention techniques and epidemiology. From 2022, a second phase of CESSARi is now focussed on transmission ecology and risk factors for cystic echinococcosis (CE) in selected areas of eastern and southern Africa.

**Results.** CE prevalence in humans and livestock was re-assessed or newly established. All five species of CE agents were found in sub-Saharan Africa. The distribution of the various taxa was found to be very uneven, and prevalence of human CE was correlated with the presence of *E. granulosus* sensu stricto transmitted in a sheep – dog cycle. Lifecycles of most *Echinococcus* species were found to involve wild animals to a previously unknown extent.

**Conclusion.** The research initiative has strengthened centres of expertise in African countries and enabled the creation of a research network across national borders. In several subjects (molecular characterization of isolates, field ultrasound scanning), training activities have shifted from European partners to African institutions, and the scientific value of the study results will in future help to acquire research funding without the crucial input from outside.

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2. Aschenborn J, Schneider C, Addy F, Aschenborn O, Kern P, Romig T, Deplazes P, Wassermann M (2022) Cystic echinococcosis of ruminant livestock in Namibia. *Veterinary Parasitology: Regional Studies and Reports* 31, 100727
3. Mulinge E, Zeyhle E, Mbae C, Gitau L, Kaburu T, Magambo J, Mackenstedt U, Romig T, Kern P, Wassermann M (2023) Cystic echinococcosis in donkeys in eastern Africa. *Parasitology* 1-8
4. Aschenborn O, Aschenborn J, Beytell P, Wachter B, Melzheimer J, Dumendiak S, Rüffler B, Mackenstedt U, Kern P, Romig T, Wassermann M (2023) High species diversity of *Echinococcus* spp. in wild mammals of Namibia. *International Journal for Parasitology: Parasites and Wildlife* 21, 134-142

## INVESTIGATING THE ORIGINS AND DISTRIBUTION OF THE EUROPEAN ECHINOCOCCUS MULTILOCULARIS ASSOCIATED WITH RECENT HUMAN ALVEOLAR ECHINOCOCCOSIS CASES IN WESTERN CANADA

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The geographic distribution of the tapeworm *Echinococcus multilocularis* (*Em*) and its prevalence in wild and domestic hosts appear to be increasing globally, either as a direct or indirect consequence of human activities. In Western Canada, genetic analyses revealed the presence of European-like strains, now circulating in wild hosts, and associated with the most recent cases of human alveolar echinococcosis (AE) in this region. As the current trend in AE incidence is raising general concerns, it is important to genetically characterize *Em* in definitive hosts to assess the current distribution and possible origins of the European strains in North America. Therefore, we aimed to *i.* characterize the genetic diversity of the *Em* in Western Canada and establish the genetic relationships with the original European clade, *ii.* assess the spatial distribution of the genetic variants, and *iii.* compare the prevalence and relative abundance of the European and North American strains within and between subpopulations of red foxes and coyotes. We collected *Em* worms from coyotes and red foxes from Alberta, Saskatchewan and British Columbia (Canada), to identify the genetic variants based on mitochondrial and nuclear markers. We found strong evidence supporting the hypothesis of a relatively recent introduction of the European strains, based on the low genetic diversity compared to that expected for a long-established endemic strain, the close genetic relationship with the original European clade, and the presence of a single predominant genetic variant in each province. Additionally, the high prevalence and intensity of infections found in coyotes support the relevant role of this host in the rapid spread of the European strains in Western Canada. These results aided to understanding the origin and eco-epidemiology of the *Em* European strains in North America, and the potential risk that the presence of these strains represents for people and domestic animals in this region.

## ОПРЕДЕЛЕНИЕ СТЕПЕНИ РИСКА ЗАРАЖЕНИЯ СЕЛЬСКОГО НАСЕЛЕНИЯ НЕКОТОРЫХ РАЙОНОВ СЕВЕРНОГО РЕГИОНА КЫРГЫЗСКОЙ РЕСПУБЛИКИ

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**Актуальность.** Результаты исследований, проведенных ранее в большинстве областей Кыргызстана, свидетельствуют о наличии риска заражения паразитарными заболеваниями, в частности эхинококкозом. Ежегодно в республике регистрируются около 1000 случаев кистозного и 200 случаев альвеолярного эхинококкоза [1]. Отрицательная динамика роста больных наблюдается в связи с ухудшением санитарно-эпидемиологического контроля и диспансеризации в животноводческих районах Кыргызстана, подобная картина наблюдается во всех странах СНГ [2].

**Цель исследования.** Определение степени риска заражения сельского населения Северного региона Кыргызской республики.

**Материалы и методы.** Анкета-опросник для сбора информации (500 штук) заполнялась методом устного опроса. Данные Национального Статистического комитета и Департамента профилактики заболеваний и санитарно-эпидемиологического надзора Кыргызской Республики. Обработка данных – IBM SPSS и EXCEL.

**Результаты.** Среди жителей сел были опрошены и заполнили анкеты 500 человек. Из них об эхинококкозе знают 74%, а 26% не знают. 86% семей в домашнем хозяйстве держат скот, а 14% – нет. Домашние питомцы (собаки, кошки) имеются у 54% семей: из которых 40% владельцы собак и кошек, а 14% имеют только кошек. 34% хозяев питомцев на привязи не держат (собаки свободно бегают по двору), а 6% – держат собак на привязи. 95 семей (19%) отметили, что дегельминтизацию животных никогда не проводили и не обращались к ветеринарам по этому поводу. 235 семей (47%) закапывают пораженные органы коров, овец и коз, 200 семей (40%) сжигают внутренние органы зарезанных животных. В 20 семьях (4%) наблюдалось скормливание собак и кошек пораженными органами скота, в 40 домах (8%) несъедобные органы животных просто выбрасывают, в 5 домах (1%) не смогли ответить на этот вопрос, т.е. они не предпринимают никаких действий. Из опрошенных в двух домах сталкивались с эхинококкозом, а в одной семье одному члену был поставлен диагноз «эхинококкоз».

**Обсуждение.** Среди опрошенных жителей сел Северного региона большинство имеют понятие об эхинококкозе и понимают, что пораженные органы следует утилизировать, а также проводить дегельминтизацию домашних животных и соблюдать правила гигиены [3]. Все же определенная часть жителей (20% и более) не имеют понятия о данном паразите. И, не понимая опасность паразита, не утилизируют пораженные органы, зачастую скормливают собак и кошек, не обращаются в ветеринарные службы.

**Выводы:** Так как основными хозяевами эхинококка являются плотоядные (собаки), заражение может происходить при непосредственном контакте с ними (собаки, шкуры волков, шакалов и т.д.) и с загрязненной внешней средой. К факторам передачи инвазии относятся почва, трава, вода из луж, арыков, содержащие яйца эхинококка.

Причиной заболевания в данной местности может быть незнание части населения о паразите, путях заражения и профилактике, а также отсутствие необходимого уровня ветеринарной службы в селе.

**Рекомендации.** Одним из основных условий, обеспечивающих защиту от ленточных червей (в частности от эхинококка) является строгое соблюдение правил личной гигиены.



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## EPIDEMIOLOGY OF CYSTIC ECHINOCOCCOSIS, A NEGLECTED WORK RELATED DISEASE IN DEVELOPING COUNTRIES

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**Relevance.** Human cystic *echinococcosis* (CE), or hydatid disease is considered as an occupational infection and among different careers with close contacts with animals are at higher risk of the infection and developing echinococcosis (1). In this research the epidemiology of CE has been compared in some developing countries (2).

**Materials and methods.** The medical publications in English and Persian electronic databases were searched from 1980 to March 2023.

**Results:** Hydatidosis rate in Iran ranging from 1.2 to 21.4 % according to serological assays mostly ELISA. The most prevalence of this disease took place in the western and southwestern areas of Iran (3).

**Conclusion.** Health educational programs are required in order to increase the knowledge and awareness of at risk groups about the disease which can be life treating. The study provides suggestions to managers and the public health authorities regarding control of the disease and prevention of spread of the infection to the human population in developing countries.

## EPIDEMIOLOGY OF ECHINOCOCCOSIS IN ISRAEL

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**Introduction.** Echinococcosis is a zoonosis caused by the larval stage of the cestode Echinococcus. E. granulosus has a worldwide geographical distribution. Infection is of feco-oral nature. Human infections are usually observed in places with poor sanitation conditions and proximity to domestic animals. In Israel, hydatid cyst disease is endemic especially among the Arab and Bedouin population in northern and southern Israel, respectively. In the Jewish population, the prevalence and incidence is much lower and we assume that most Echinococcosis cases were acquired in their country of origin. Up to date, no comprehensive epidemiological surveys of the disease in Israel, were performed.

**Objective.** The primary objective was to estimate disease frequency in the Israeli population. The secondary was to collect clinical data of the disease.

**Methods.** A retrospective study of Echinococcosis in Israel during the years 1995-2017. Data was collected from multiple databases. Data about hospitalized patients due to Echinococcosis, by ICD-9 relevant coding, was collected from the MOH Data department. Serology diagnosis of Echinococcosis was retrieved from the Parasitology laboratory of MOH and from Soroka medical center. For more clinical information we collected data about hospitalized patients at our center-Sheba Medical Center (SMC).

**Results.** During these years there were a total of 797 hospitalized patients due to Echinococcosis, with a prevalence of 0.2-1.2 per 100,000 residents per year and an average of  $0.6 \pm 0.29$ . Prevalence was clearly declined throughout the study years.

In the years 2010-2017 there were 330 positive serology cases; confirmed ( $n=166$ ) and probable ( $n=164$ ) cases, by Western blot and ELISA, respectively. To this date we were unable to assess the extent of overlap between these two databases.

During the years 1995-2017, 113 patients with a history of Echinococcosis (either primary or secondary diagnosis) were hospitalized In Sheba Medical Center. Most of the study population were Jews (107/113) and the rest (6 patients) were Arabs. Most of the Jewish patients were actually born abroad (99/107), and therefore only 8/107 cases in Jews and all 6 cases in Arabs were autochthonous. Jewish population born abroad originated mainly from Middle-East Arab countries (52.9%), Europe (mostly Eastern) (21.2%), and North and East Africa (12.5%).

There were 38 patients hospitalized primarily due to Echinococcosis at SMC. These patients had 71 hospitalizations, with a mean of 1.8 hospitalizations/patient (range of 1-10). Most hospitalizations were in the Surgical ward (80.5%) and the rest were in the Internal medicine ward for diagnosis or conservative therapy. About third of the patients were not cured by the first surgery (31.5%) and underwent a second surgery. The frequency of post-operative complications was high (19%).

**Conclusions.** This is the first comprehensive study regarding prevalence of Echinococcosis in Israel. The disease is not uncommon but seems to decline over the study years. In Jewish patients, most cases were acquired abroad. The disease required multiple hospitalizations. Surgical treatment was common but with a significant rate of complications and need for second surgery. Thus, an effective and safe treatment is highly needed for this disease.

## ECHINOCOCCOSIS IN BHUTAN

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Environmental contamination of *Echinococcus* eggs were detected in the towns and in yak rearing areas as per studies conducted from 2012 to 2021[4-5]. *Echinococcus* cysts were documented in cattle [2]. Large canine populations (56269 stray dogs and 31622 owned dogs) cohabitation with the human population pose a zoonotic risk in transmitting echinococcosis.

The environmental faecal samples and soil samples were processed using (F/Si) [1] at the National Center for Animal Health, Serbithang, Thimphu, Bhutan; DNA extraction was carried out as described [3] and sequencing using multiplex PCR as described by [6] at Institute of Parasitology, Zurich, Switzerland.

Year	Areas	Samples examined	Positive (microscopically)	Taeniid species
2011	Impounded stray dogs	338	18(5.3%)	4 <i>E. granulosus</i> s.l
2012	Thimphu city	138	20 (14.4%)	10 <i>E. granulosus</i> s.s. and one <i>T. hydatigena</i>



2012	Carnivores around farm, central Bhutan	28	14(51.4%)	one <i>E. granulosus</i> s.l., six <i>T. hydatigena</i> and one <i>Hydatigera taeniaeformis</i>
2016-2018	Nation wide (20 districts)	953	67(7%)	22 (3.2%) <i>E. granulosus</i> s.s. and four (0.5%) as <i>E. ortleppi</i> (G5)
2016-2018	Yak grazing areas	283	27(9.5%)	including eight (2.8%) with <i>E. granulosus</i> s.s. and four (1.4%) with <i>E. ortleppi</i> . <i>E. granulosus</i> s.s.
2016-18	Mithun cyst	1	1	<i>E. granulosus</i> s.s.

Findings suggested, potential dog-human transmission of *E. granulosus* s.s. [3]. Hospital records document human cystic echinococcosis in Bhutan and *E. granulosus* s.s. is the common species followed by *E. ortleppi* found in the dog population in Bhutan.

### THE BURDEN AND DISTRIBUTION OF CYSTIC ECHINOCOCCOSIS IN BHUTAN: A RETROSPECTIVE STUDY

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**Background.** Cystic echinococcosis (CE) is prevalent in most continents posing a significant public health challenge. This study was conducted to gain an understanding of the burden, distribution, and potential risk factors of CE in Bhutan.

**Method.** Data from five years (2015-2019) from the National Referral Hospital and six other district-level hospitals were reviewed and analyzed.

**Result.** The majority (145) were treated in the country and 14 were referred to India. The average annual incidence was 4.4 cases per 100,000 population. Wangdue and Bumthang had the highest proportion of cases. Females, farmers, and those aged 30-59 years had the higher notification rates. The liver (77.98%) and lungs (13.21%) were the commonest sites of infections. Most received surgical treatment (>82%), and more than 47% were admitted for >4 days.

**Conclusions.** The findings suggest a high incidence of CE in the country. Policy interventions guided by more studies may yield larger gains.

### 40 YEARS OF EXPERIENCE OF CONTROL PROGRAM OF CYSTIC ECHINOCOCCOSIS OF RIO NEGRO PROVINCE, ARGENTINA

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The Ministry of Health of Rio Negro Province launched the Control Program of Cystic Echinococcosis (CE) in 1980 with ONE HEALTH concept to overcome the problem of human CE in Rio Negro with a high incidence (45/100000), high mortality (5%) and 5.6% of school children with hepatic CE.

For primary prevention teachers, community health workers, nurses and veterinarians working in health education focused on: avoiding offering raw offals to dogs, systematic deworming dogs with praziquantel, and recently in a selected area, using lamb's vaccination. For surveillance for many years arecoline test was used and now is done by coproantigens study of dog's fecal samples by coproELISA.

For secondary prevention, screening has done with serology until 1996 by ELISA test. Since 1997 is done with Focused Assessment with Sonography for Echinococcus (FASE) a point of care ultrasound (POCUS) approach focused on schoolchildren, an original developing of Rio Negro Control Program of CE. This is for early diagnosis, follow up and for monitoring the program. A new case in children prove recent transmission.

For tertiary prevention, children positive in FASE mostly are asymptomatic, and can be treated successfully up to 90% with albendazol and only 10% of them (if cyst growing or become symptomatic) will need an invasive treatment.

The continuity of the program over the years lead to drop the incidence to 4/100000 and 0.1% of school children with hepatic CE. Despite having maintained the measures to control with the aim to eradicate CE, transmission is still present with new cases in school children every year.

## **EPIDEMIOLOGICAL FEATURES AND SPATIAL CLUSTER ANALYSIS OF HUMAN ECHINOCOCCOSIS IN SOUTHERN XINJIANG FROM 2005 TO 2021**

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**Objectives.** Human echinococcosis remains an important public health problem. The aim of this study was to investigate the epidemiological features and Spatial cluster analysis of human echinococcosis in southern Xinjiang, China from 2005 to 2021.

**Methods.** In this study, data on reported echinococcosis cases and incidence from 2005 to 2021 were obtained from the National Infectious Disease Reporting System. Joinpoint regression analysis was performed to explore the trends of echinococcosis in southern Xinjiang. The global spatial autocorrelation statistic (Moran's *I*) and the local indicators of spatial association (LISA), as well as hot spot analysis, were conducted to identify the distribution and risk areas of echinococcosis.

**Results.** A total of 4581 echinococcosis cases were reported in southern Xinjiang, China during 2005–2021, with a mean annual incidence of 2.56/100,000. Echinococcosis incidence showed a decreasing trend in general during our study period (average annual percent change [AAPC]= 7.444, 95% confidence interval [CI]: 3.650 to 11.376). The number of reported cases of echinococcosis in Southern Xinjiang is primarily among farmers between the ages of 40 and 50, with no significant gender difference. Hejing County has the highest prevalence, while Minfeng County has the lowest. echinococcosis cases had a positive global spatial auto-correlation in 2005-2021 (Moran's *I* =0.21, *P*<0.05), evident by high-high and low-low clustering areas and hotspots. Clustering is primarily localized in the northeastern region of Southern Xinjiang.

**Conclusions.** Our findings suggest that echinococcosis is still an important zoonotic parasitic disease in southern Xinjiang, China. Epidemic trends in different counties (cities) varied degrees

significantly, yet showed a certain degree of spatial clustering. Mitigation strategies, including large-scale screening, reinforcing dog management practices, conducting health education campaigns, and combining other comprehensive methods are needed to reduce transmission risk in areas at risk.

**Key words:** Echinococcosis, Epidemiology, Xinjiang, Spatial Clustering

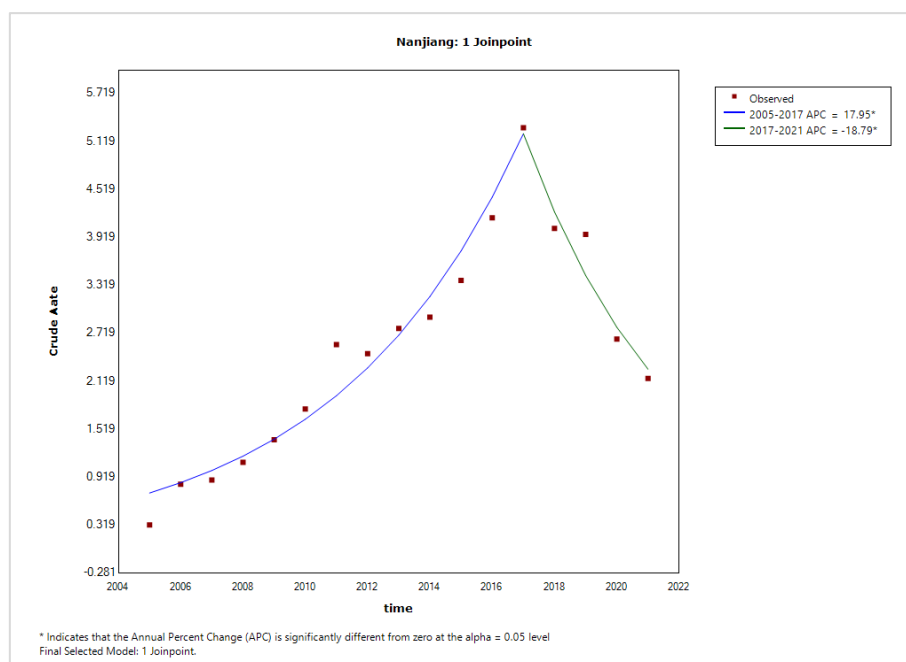


Figure 1. The joinpoint regression analysis for determining changes in the trend of echinococcosis incidence in southern Xinjiang, China from 2005 to 2021.

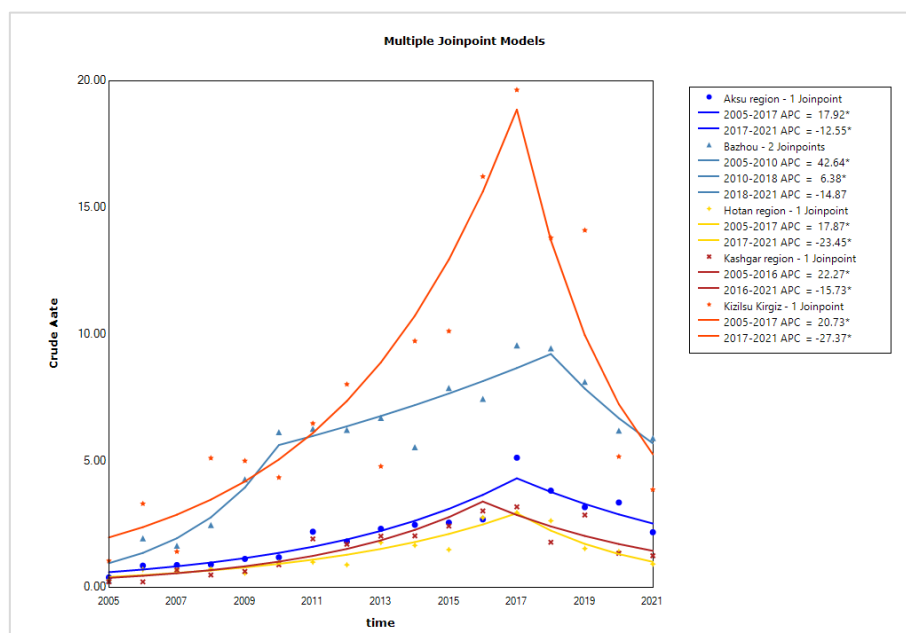


Figure 2. The joinpoint regression analysis for determining changes in the trend of echinococcosis incidence in different regions (prefectures) of southern Xinjiang, China from 2005 to 2021.

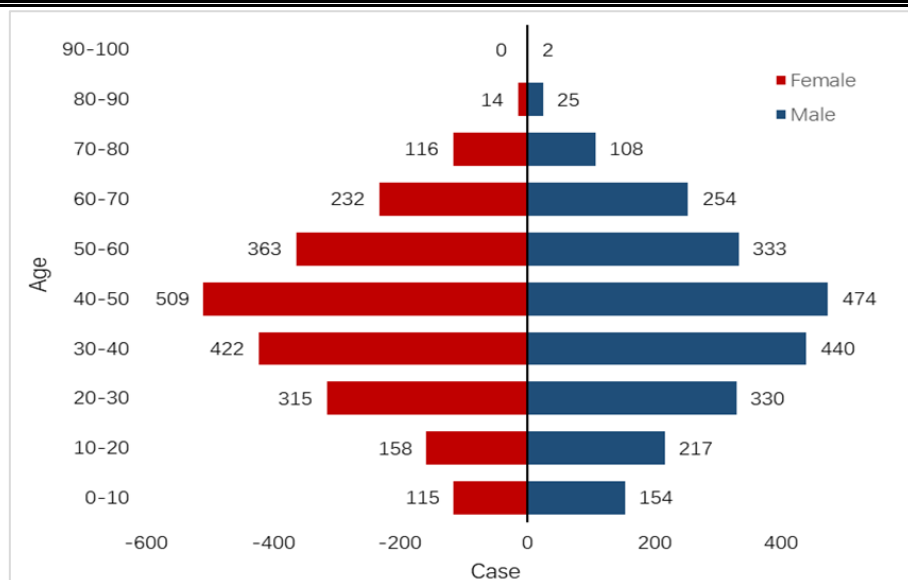


Figure 3. Prevalence of echinococcosis in different age groups of men and women in southern Xinjiang, China from 2005 to 2021.

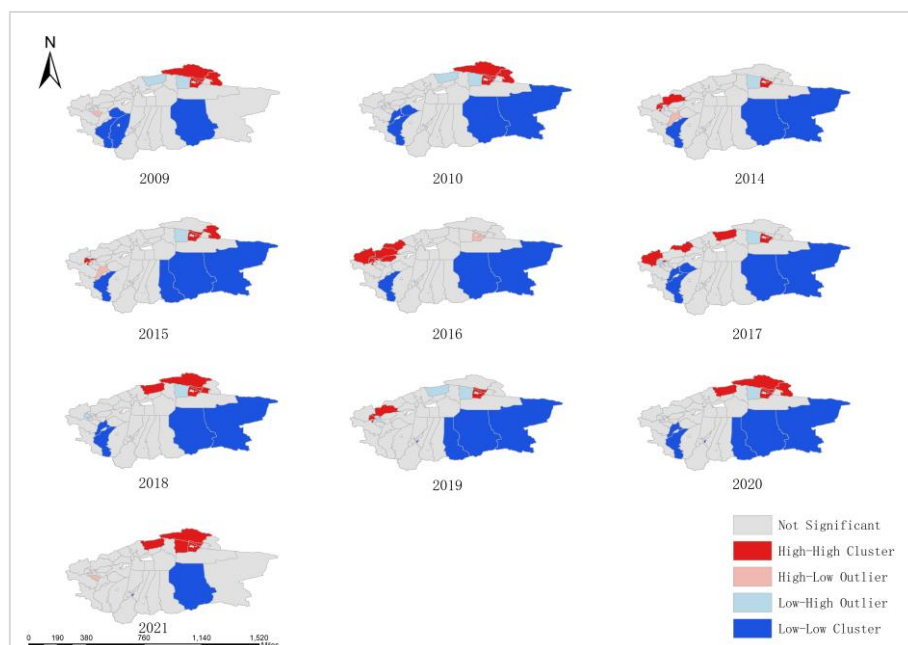


Figure 4. Local indicators of spatial association of echinococcosis in southern Xinjiang in 10 representative years.

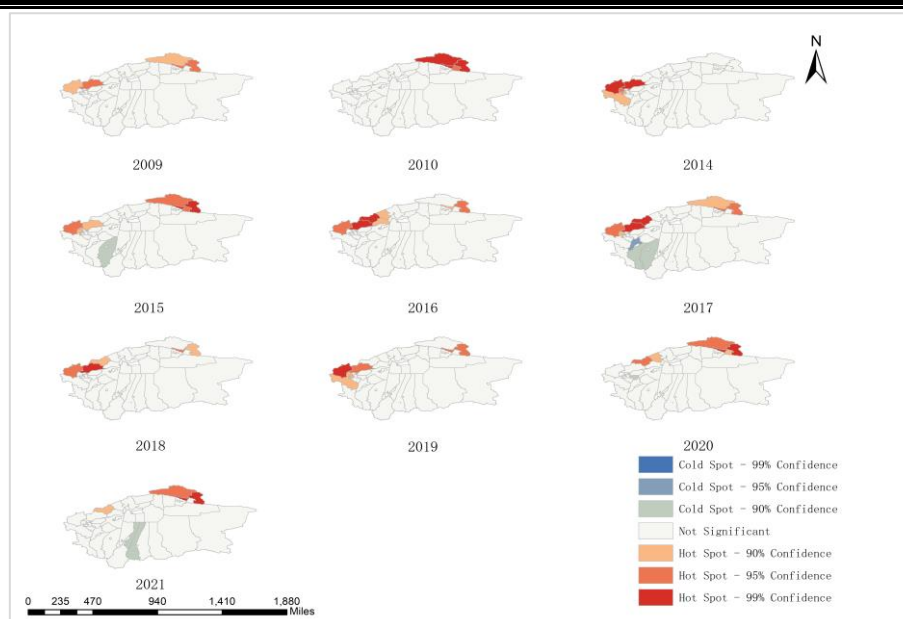


Figure 5. Hotspot analysis of echinococcosis incidence in southern Xinjiang from 2005 to 2021.

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## IS THE RISK OF ALVEOLAR ECHINOCOCCOSIS HIGHER IN RURAL OR URBAN AREAS?

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**Aim.** The aim of this study was to evaluate the dynamics of *E. multilocularis* in Erzurum by taking into account the results of the research carried out on the final and intermediate hosts.

**Material and Method.** In the project, 50 fox, 2 wolf, and 2 lynx carcasses, 600 foxes, and 446 stray dog feces were examined. The risk status for the spread of AE in rural and urban areas of the province was assessed on the basis of final and intermediate host infectivity.

**Result.** *E. multilocularis* was detected in 42% (21/50) of fox carcasses, 50% (1/2) of wolf carcasses and 50% (1/2) of lynx carcasses, 10.5% (63/600) of fox feces, and 3.58% (16/446) of dog feces. The intensity of infection in both the carcass and fecal samples was high in the city center.

**Conclusion.** According to these results, AE, which is thought to have a sylvatic cycle, is more common in areas close to the city center. Stray dogs act as a bridge between rural and urban life.

## ONE HEALTH IN CENTRAL ASIA: STATUS QUO AND FUTURE STEPS

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One Health approach is a comprehensive and proactive framework that considers the interplay between human, animal and environmental factors in disease emergence and control. It brings together governments, healthcare professionals, veterinarians, researchers and communities to combat health issues. Central Asia historically has been endemic to multiple environmental threats, different types of zoonosis and environmental catastrophic events, such as Aral Sea recession. During Soviet Union Central Asian countries had zoonosis surveillance system that was connecting all countries, however after its fall Central Asian countries had fragmented initiatives in One Health.

Therefore, we started ERASMUS+ project HARMONEE that lead to joint Central Asian master programme development. Consortium consists of nine Central Asian Higher Education Institutes in four different countries and two European Universities.

Based on a holistic perspective master education was developed by using a Teach the Teacher approach for a sustainable output. This project resulted in creation of new modules for strengthening of disciplinary skills required in the field of One Health.

Building capacity and creating One Health network in Central Asia will help create a form of researchers, policy makers and professionals to tackle future environmental threats in the region.

PREVALENCE AND DISTRIBUTION OF ECHINOCOCCOSIS  
IN TACHENG, XINJIANG, 2005-2020**Shi Guangzhong, Yang Hanqi**Xinjiang Uygur Autonomous Region Center  
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**Abstract.** The Tacheng region is located in the northwest of Xinjiang, located in the central part of Ili Kazakh Autonomous Prefecture, belonging to the arid and semi-arid climate areas of the temperate zone. It governs Tacheng City, Shawan City, Wusu City, Emin County, Yumin County, Toli County, Hoboksar Mongol Autonomous County, the local economic structure is relatively single, mainly focusing on agriculture and animal husbandry. It is a highly prevalent area for echinococcosis.

**Objective.** To understand the prevalence and distribution of echinococcosis in Tacheng, Xinjiang Uygur autonomous region, from 2005 to 2020, and provide evidence for the local prevention and treatment of echinococcosis.

**Methods.** The data of echinococcosis cases reported in Tacheng from 2005 to 2020 were collected from national infectious disease reporting information management system. Excel 2007 and SPSS 17.0 were used to analyze the distribution of echinococcosis cases by area, age, gender and population.

**Results.** A total of 2 356 cases of echinococcosis were reported in Tacheng from 2005 to 2020. The average annual incidence was 14.39/100 000, and the highest incidence was 29.27/100 000 in 2017. There was a statistical difference in the quarter distribution of the cases ( $\chi^2 = 172.75$ ,  $P < 0.05$ ), the number of reported cases in the first and fourth quarters was significantly higher than that in the second and third quarters; The gender ratio was about 1.09:1, and there was no significant difference



in gender distribution of the cases ( $\chi^2=11.88$ ,  $P>0.05$ ). The age of the cases ranged from 2 to 90 years with an average age of 38 years. The cases in farmers and herdsmen (1 416 cases) accounted for the highest proportion (60.10%). The cases were reported in Tacheng, Emin, Yumin, Toli, Wusu, Shawan, and Buxel. The annual average incidence in Toli was highest (45.80/100 000), where 436 cases were reported cumulatively, accounting for 18.51% of the total, the differences in incidence among different areas were significant ( $\chi^2=311.98$ ,  $P<0.05$ ). Among the reported cases, 2 163 (91.81%) were reported by hospitals.

**Conclusion.** The overall incidence of echinococcosis in Tacheng showed an increase trend from 2005 to 2020 although decline occurred after 2017. The echinococcosis cases were mainly distributed in young adults and adults engaged in agriculture and aquaculture in pastoral areas and semi-agricultural and semipastoral areas. In the prevention and control of echinococcosis, it is necessary to strengthen the health education about echinococcosis in key populations and adhere to the mode of integration of prevention and treatment for the early detection, diagnosis and treatment of echinococcosis.

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## POLYCLONAL AND MONOCLONAL ANTIBODIES BASED COPRO-ELISA FOR DETECTING ECHINOCOCCUS GRANULOSUS ANTIGENS IN DOG FECAL SAMPLES

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**Relevance.** Cystic echinococcosis (CE), caused by the metacestode *Echinococcus granulosus sensu stricto* (s.s.), is an important zoonotic parasite. We established an ELISA method with high sensitivity and specificity for the detection of *Echinococcus granulosus* antigen in dogs.

**Materials and methods.** New Zealand white rabbits were immunized with *Echinococcus granulosus* surface membrane antigen (EgSfAg) to prepare polyclonal serum. Monoclonal antibody against EgSfAg was prepared. Polyclonal antibodies and monoclonal antibody 4E8 were purified by chromatography. Antigens from five species tapeworms were prepared and used as specific quality control samples, including *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia vesiculata*, *Taenia vesicularis*, *Taenia polycephalus*, *Toxocara canis*. The positive control samples and negative control samples were prepared by artificially infected dogs. An ELISA method was established using polyclonal antibody as capture antibody, and HRP-labeled monoclonal antibody 4E8 as detection antibody. The sensitivity and specificity of the ELISA method were verified.

**Results.** The sensitivity of 130 positive fecal samples was 95.38%. Cross-experimental results showed only EgSfAg was positive, no cross-reaction with antigens from other tapeworms. The specificity of the test for 150 non-*Echinococcus* samples was 95.56 %.

**Conclusion.** The established polyclonal and monoclonal antibodies based coproELISA was sensitive with high specificity, which provides a safe, simple and efficient immunological detection method for detecting *Echinococcus* in dog fecal samples.

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## VASCULAR PATHOLOGY IN PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS: FRAMEWORK FOR ASSESSMENT AND CLINICAL MANAGEMENT – A RETROSPECTIVE CASE SERIES

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**Objective.** Alveolar echinococcosis (AE) is a parasitic liver disease with infiltrative growth similar to solid organ malignancies. Major vascular damage is frequent and often remains untreated until catastrophic events precipitate. Detailed clinical and radiological assessment is required to guide individualized treatment decisions. Standardized radiological reporting templates of malignancies with profiles resembling AE are candidates for adaptation. Our objectives are to describe vascular pathology in AE and establish a framework for structured evaluation as the basis for treatment decisions and monitoring.

**Design.** Retrospective case series.

**Results.** 69 patients (37.1%) had vascular involvement: portal vein (PV) 24,7%, hepatic vein (HV) 22,6% inferior vena cava (IVC) 13,4%. Significant stenosis/occlusion of vessels was present in 15,1% of PV, in 13,4% of HV and in 7,5% of IVC involvement. Vascular pathology needing specific treatment or monitoring was present in 8,6% of patients. The most frequent clinical presentation was high grade IVC-stenosis or occlusion which was seen in 11 patients of the cohort.

**Conclusion.** Advanced AE requires early multidisciplinary assessment to prevent progressive impairment of liver function due to vascular damage. The focus at first presentation is on complete evaluation of vascular (and biliary) involvement. The focus in non-resectable AE is on prevention of vascular (and biliary) complications whilst suppressing growth of AE lesions by benzimidazole treatment to improve the quality of life of patients. We developed a framework for standardized vascular assessment and follow up of AE patients to recognize and treat complications early.

## PRIMARY CEREBRAL CYSTIC ECHINOCOCCOSIS IN A CHILD FROM ROMAN COUNTRYSIDE: SOURCE ATTRIBUTION AND SCOPING REVIEW OF CASES FROM THE LITERATURE

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Human cystic echinococcosis (CE) is a zoonotic parasitic infection caused by the larval stage of the species belonging to the *Echinococcus granulosus sensu lato* (s.l.) complex. Parasitic cysts causing human CE are mainly localized in the liver and in the lungs. In a smaller number of cases, larvae may establish in any organ or tissue, including the central nervous system (CNS) [1].

This study presents a case of CCE in a child living in the countryside near Rome (Italy), along with a comparative molecular analysis of the isolated cyst specimens from the patient and sheep of

local farms. The comparative molecular analysis confirmed that the infection was caused by *E. granulosus sensu stricto* (s.s.) (G3 genotype) [2].

We also summarized the clinical characteristics of CCE cases retrieved through a literature review. When considering clinical centres reporting all anatomical sites of CE, liver represented 70%, lungs 19%, and unusual localizations 11% of all CE cases. The proportion of CCE among all CE cyst localizations represented 1.5% of all CE cases. The literature search identified 2,239 cases of CCE. In 80.51% of cases, brain was the only localization and single CCE cysts were present in 84.07% of cases. Mean patients' age was 20 years and 70.46% were children. In the few reports that identified at molecular level the CCE cyst, *E. granulosus* s.s. was found in 40% and *E. canadensis* in 60% of cases. We reported a rare case of CCE and evidenced the probable local origin of infection. The proportions of CE cases with uncommon localizations and with high impact on patients' lives have been globally neglected and should be included in the computation of the global burden of CE.

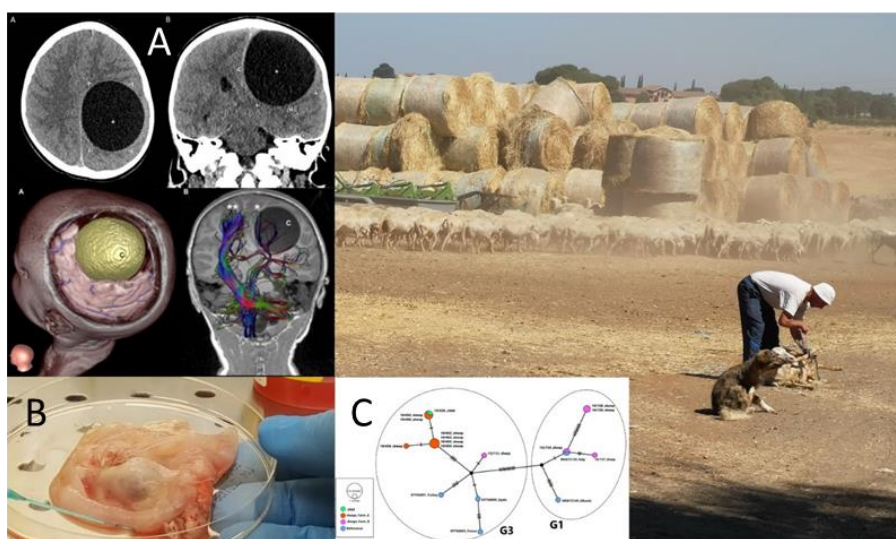


Figure1. A) Computed tomography imaging of the cerebral echinococcal cyst and tridimensional reconstruction of the cystic lesion.  
B) Cerebral echinococcal cyst removed from the patient.  
C) Haplotype network of the concatenated sequences of the mitochondrial genes COX1, NAD5, NAD2 and NAD1.

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## CHALLENGES IN THE DIAGNOSIS AND MEDICAL MANAGEMENT OF ALVEOLAR ECHINOCOCCOSIS IN EUROPE

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Alveolar echinococcosis (AE) is an orphan zoonotic liver disease of increasing concern in several part of Europe. The diagnosis is still challenging, leading often to a delay in diagnosis of several months. According to the WHO-IWGE diagnostic criteria, the diagnostic certainty is classified as **possible** (clinical and epidemiological history and imaging findings **or** serology positive for AE), **probable** (clinical and epidemiological history and imaging findings **and** serology positive for AE with two tests, and **confirmed** (the above, plus histopathology compatible with AE and/or (2) detection of *E. multilocularis* nucleic acid sequence(s) in a clinical specimen. Imaging (ultrasound with or without contrast), CT, MRI and PET) plays a crucial role in the initial diagnosis of the disease. For each modality different classification scheme have been proposed. Nevertheless, the differential diagnosis from primary and secondary liver tumors remains challenging. If artificial intelligence will help to improve the diagnostic accuracy of imaging remains to be seen. In this setting AE serology might be helpful. Different in house and commercial tests are available with sensitivities and specificities ranging between 91-96% and 97-99% respectively for the best assays. However, the diagnostic accuracy of the test in patients with small lesion was evaluated only in a limited number of patients. In situation where the diagnosis remains unclear, liver histology and/or PCR for *E. multilocularis* can be extremely helpful. Careful evaluation of conventional histological findings such as size of smallest (CE/AE:  $>2/\leq 2$  mm) and largest cyst (CE/AE:  $>25/\leq 25$  mm), thickness of laminated layer (CE/AE:  $>0.15/\leq 0.15$  mm) and pericystic fibrosis (CE/AE:  $>0.6/\leq 0.6$  mm), striation of laminated layer (CE/AE: moderate-strong/weak), and number of cysts (CE/AE:  $\leq 9/>9$ ) allow to discriminate between CE and AE. This can be supported by immunohistochemistry with two antibodies: mAbEm2G11, which is *E. multilocularis* specific, and mAbEmG3, which is reactive in AE and CE.

Medical treatment with Benzimidazols, today mostly albendazole, has dramatically improved the prognosis of patients with AE over the last half century. Several European cohort reported excellent long-term survival even in inoperable patients with AE. In recent years, it was recognized, that even in this patient population a structured treatment interruption is possible in a highly selected group of patients. A further refinement of this patient group with new biomarkers is desirable. On the other hand, not all patients tolerate albendazole, even after reducing the dose and carefully adjusting based therapeutic drug monitoring. In this situation, a switch to mebendazole might be necessary, again carefully monitoring the dose based on drug monitoring. Apart from benzimidazoles, there are no approved alternative drugs available. New drugs, ideally parasitocidal, are urgently needed.

### EFFECT EVALUATION AND FACTOR ANALYSIS OF ULTRASONIC MANIFESTATIONS IN THE DIAGNOSIS OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

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**Relevance.** To explore the influence of different ultrasound manifestations on the ultrasonic diagnosis of hepatic alveolar echinococcosis by univariate and multivariate Logistic regression analysis, so as to provide a reliable basis for the diagnostic accuracy of hepatic alveolar echinococcosis.

**Materials and methods.** Retrospective analysis of ultrasound data of patients with liver multilocular echinococcosis who visited our hospital from January 2018 to April 2021 and underwent routine ultrasound examination before surgery. The univariate analysis was performed first, and then the meaningful indicators were analyzed by multivariate Logistic regression analysis, and the regression model was established. The ROC curve was drawn to predict its accuracy; then, the nomogram was



constructed by analyzing the factors affecting the diagnostic coincidence, and the ROC curve, calibration curve and decision curve (DCA) were established to evaluate its predictive performance.

**Results.** Univariate analysis showed that the size, boundary and shape, internal echo, calcification, liquefaction necrosis, blood flow signal, bile duct and blood vessel invasion of the lesion had an effect on the coincidence rate of ultrasound diagnosis, and the difference was statistically significant ( $P < 0.05$ ). Multivariate Logistic regression analysis showed that size, calcification, liquefaction necrosis, blood flow signal were correlated with ultrasound diagnosis ( $P < 0.05$ ). The sensitivity of ultrasound diagnosis was 96.7%, the specificity was 83.3%, and the area under the ROC curve was 0.918 (95% CI : 0.859 ~ 0.977). Based on the results of multivariate Logistic regression analysis, a nomogram was constructed, and its excellent performance was verified by ROC curve, calibration curve and decision curve (DCA).

**Conclusion.** The size, calcification, liquefaction necrosis and blood flow signal of hepatic alveolar echinococcosis have an effect on the results of ultrasonic diagnosis, which can provide a reliable basis for the accuracy of ultrasonic diagnosis of hepatic alveolar echinococcosis.

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## INVESTIGATION OF PATIENTS WITH SECONDARY INFECTION OF ECHINOCOCCAL CYST IN SARDINIA, ITALY

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**Background.** Cystic Echinococcosis (CE) is a zoonotic disease caused by the larval stage of a tapeworm that belongs to the genus *Echinococcus* and species *Echinococcus granulosus sensu lato* (*s.l.*). CE is a worldwide public health problem and is highly incident in all Mediterranean areas (Eckert et al., 2001). Furthermore, after a certain period of time, several subjects may manifest the onset of a new cyst named secondary cyst (Velasco-Tirado et al., 2017). The purpose of our survey was to deeply investigate 20 patients, out of the 83 enrolled in the study, which presented a secondary echinococcal cyst.

**Materials and methods.** A diagnosis for CE was done for the 83 subjects involved in the this report, comprising 36 (43.37%) females and 47 (56.63%) males, by means of clinical, imagine techniques (ultrasound, radiography, magnetic resonance, computed tomography) and serological findings [immunochromatographic test VIRapid Hydatidosis (Viracell) and Echinococcus Western Blot IgG (LDBIO)]. Mean age at diagnosis was 50.71 years (SD ± 20.13). Five patients, 3 males and 2 females, were from China, Ghana, Pakistan, Romania and Morocco, but resident in Sardinia (Italy) at the time of diagnosis. Their general and medical data were entered into a database and evaluated.



**Results and Discussion.** According to the data recorded on the database, 20 (24.10%) patients developed a secondary cyst. Percentage found in this survey were higher respect to those reported in other countries as Greece (8.7%), Turkey (9.45%), Spain (11.5%). In this small group, the majority were males (n=13) respect to females (n=7) and the mean age of at diagnosis was 41.3 years (SD  $\pm$  18.41). Concerning stadiation of the primary lesions of these 20 patients, all stages were represented: CE1 (n=3), CE2 (n=6), CE3a (n=2), CE3b (n=2), CE4 (n=6) and CE5 (n=3) and 5 unstaged. While, stages and numbers of secondary lesions corresponded respectively to CE1 (n=2), CE2 (n= 5), CE3a (n=2), CE3b (n=8), CE4 (n= 7) and CE5 (n=4), being the stage unknown for 4 patients. In patients with secondary infection the number of lesions has increased (n=35) respect to the cysts of primary infection (n=28). Moreover, multiple cysts were also augmented, and a high percentage of lesions in organs different from liver was also observed, finally, a major mortality rate was observed. Furthermore, 2 patients developed 2 secondary lesions: one had the first manifestation after 4 years (CE3b) and the second after 25 years (CE3b) the other subject developed the secondary lesion after 2 (CE2) and 6 (CE3b) years. The treatments of primary infection for the 20 patients corresponded to: traditional surgery for 7 patients; ABZ for 6 subjects; PAIR in 3 patients; no medical therapy for 3 subjects, 4 underwent to Watch and Wait, 1 subject not received any medical therapy, finally, for 1 patients any data was available.

**Conclusion.** Our analysis evidence that majority of primary cyst in patients with a secondary infection were treated by surgery, moreover, they presented a more severe diagnostic picture comprising an increased number of patients, lesions and dimension of cysts.

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## ULTRASOUND DIAGNOSIS OF CE: UPDATES AND IMPLICATIONS FOR CLINICAL MANAGEMENT

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**Relevance.** The diagnosis of cystic echinococcosis (CE) is based on imaging [1]. The recognition of a focal lesion with morphological characteristics of *Echinococcus granulosus sensu lato* metacestode is the starting point for any diagnostic workup. In organs explorable with ultrasound (US), this is the method of choice for both etiological diagnosis of CE and staging of the CE cyst [2], since this imaging method is able to reliably depict the morphological characteristics, which are pathognomonic of the metacestode and of its stage. Staging in terms of evaluation of morphology by US is also needed when serology is added to the diagnostic workflow in case imaging is inconclusive, since interpretation of serology results has to be performed in the light of the possible CE stage [3]. Finally, especially in the liver, staging guides the clinical management of uncomplicated CE; therefore no up-to-standard clinical management can be implemented in the absence of correct staging of the CE cyst.

Despite the importance of imaging and of staging for the correct diagnosis and management of CE, and the availability since 2010 of an Expert Consensus [1], still misdiagnosis and mismanagement are extremely frequent and even published.

Currently, the WHO-IWGE is carrying out two pivotal activities to provide the scientific and healthcare community with practical and updated guidance on the diagnosis and clinical management of CE, based on scientific evidence available. These are the production of a consensus diagnostic algorithm and case definitions, and the issue of official WHO guidelines for the clinical management of CE.

This keynote will provide an overview of the most updated evidence backing the above-mentioned role of US in the diagnosis and clinical management of CE, and will outline current activities of the WHO-IWGE, which will be the object of dedicated presentations during the congress

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## CONSENSUS ON THE CASE DEFINITIONS AND DIAGNOSTIC ALGORITHM FOR CYSTIC ECHINOCOCCOSIS: A DELPHI STUDY

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**Relevance.** The diagnosis of cystic echinococcosis (CE) is based on imaging, complemented by serology. The 2010 WHO-IWGE Expert Consensus on clinical management of echinococcosis [1] provides some diagnostic guidance, but a widely agreed standardized diagnostic algorithm is not available. Defining "confirmed" and "suspected" CE is key for clinical practice and scientific reporting. The WHO-GDG "Echinococcosis" is currently working on clinical management guidelines for CE. The **aim** of this Delphi study is to achieve consensus on a diagnostic algorithm and case definitions of CE, to support and complement the WHO guidelines.

**Materials and Methods.** The study uses the Delphi methodology [2] to reach and "Expert"-based consensus and the REDCap online platform. WHO-IWGE members experienced in the diagnosis and management of CE and a sample of staff of selected parasitology labs ("Experts") were invited to participate. Statements on the diagnosis of CE based on current scientific literature [e.g. 3,4], tentative CE case definitions and diagnostic algorithm, are submitted to "Experts" for agreement ranked on a Likert's scale. In cases of disagreement, the "Experts" are asked to provide an alternative statement and supporting arguments, preferably published evidence. Ranking of each statement is evaluated for level of consensus, and re-formulated to account for the feedback and re-submitted to "Experts". A cascade decision system is used to define when consensus is reached and to terminate the survey. The "Experts" feedback is analyzed anonymously and "Experts" identities are concealed.

**Results.** Excluding study coordinators, 38 “Experts” were invited to Round-1, of whom 26 (68%) replied. Agreement thresholds were reached for 25 of 29 statements. Interim results will be presented.

**Conclusion.** It is expected that clear and standardized guidance on the diagnostic process and CE case definitions will improve diagnosis and clinical management of CE, and scientific reporting.

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## THE VALUE OF NOMOGRAM BASED ON IMAGING AND CLINICAL FEATURES TO PREDICT HEPATIC ALVEOLAR ECHINOCOCCUS LUNG METASTASIS

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**Objective.** Hepatic alveolar echinococcosis (AE) of liver grows in a way similar to malignant tumor, and has a tendency of invasive growth and metastasis. When lung metastasis occurs, the prognosis is usually poor. To investigate the value of nomogram based on CT and MRI imaging features combined with clinical features in predicting pulmonary metastasis of alveolar echinococcosis.

**Methods.** CT and MRI Imaging features and clinical features of 297 patients diagnosed by pathology from 2015 to 2022 at the Affiliated Hospital of Qinghai University were collected retrospectively. They were randomly divided into training set (208 cases) and test set (89 cases) according to 7:3, and the chi-square test or t-test was used for comparison between two groups. Univariate and multivariate logistic regression analyses were used to obtain independent predictors of AE pulmonary metastasis. Based on this, a prediction model for AE lung metastasis was constructed and expressed as a nomogram, and the model performance was evaluated using receive operating characteristic (ROC) curves and calibration curves, and the clinical value of the prediction model was assessed using decision curve analysis (DCA).

**Results.** The CT and MRI imaging characteristics and clinical indicators showed no significant differences between the training and test sets ( $p > 0.05$ ). After univariate and multivariate logistic regression analysis, it was found that lesion size, cystic type, enhancement form, treatment mode and other metastasis were independent predictors of AE lung metastasis. The nomogram model was constructed based on these five variables. The area under ROC curve (AUC), cutoff value, sensitivity and specificity of training set and test set are 0.822 and 0.773, 0.4813 and 0.4469, 97.37 and 92.31, 50.76 and 52.38 respectively. The calibration curve shows that there is a good consistency between the actual and predicted probability of the sample. DCA shows that the model has high clinical value.

**Conclusion.** The model based on the lesion size, cystic composition, enhancement form, treatment style, and other metastases can be used to predict the lung metastasis of AE, which is helpful to grasp the progression status of the disease and can guide the individualized treatment to a certain extent.

## THE VALUE OF MRI FUNCTIONAL IMAGING NOMOGRAM IN DIFFERENTIATING CEREBRAL ALVEOLAR ECHINOCOCCOSIS FROM BRAIN METASTASES

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**Objective.** Cerebral alveolar echinococcosis (CAE) is extremely rare and is mainly caused by late metastasis of hepatic alveolar echinococcosis, which is very similar to brain metastases (BM) in clinical and imaging manifestations. To investigate the value of MRI functional imaging nomogram based on diffusion kurtosis imaging (DKI), three-dimensional arterial spin labeling (3D-ASL) to differentiate CAE from BM and to evaluate their efficacy and clinical application value.

**Materials and methods.** Twenty-four patients (86 lesions) with CAE and 16 patients (69 lesions) with BM were collected at Qinghai University Affiliated Hospital from 2015 to 2022, which were pathologically confirmed or comprehensively diagnosed. Both groups were scanned by DKI and ASL, and DKI parameters (Kmean, Dmean, FA, ADC) and cerebral blood flow (CBF) were analyzed in the parenchymal, edematous, and symmetrical normal brain tissue regions of the lesions in the two groups, and all the parameters of the parenchymal and edematous regions of the lesions were compared with those of the contralateral normal brain tissues in order to obtain standardized parameter values. A total of 155 lesions in the two groups were divided into a training set (108 lesions) and a test set (47 lesions) according to 7:3, and comparisons between the two groups were made using the Independent samples t-test, the rank sum test, or the chi-square test. Univariate and multivariate Logistic regression analysis was used to obtain independent predictors for the identification of CAE and BM. Based on this, a predictive model was constructed and expressed in the form of a nomogram. Receiver operating characteristic (ROC) curves and calibration curves were used to evaluate the performance of the model. The clinical value of the predictive models was assessed using decision curve analysis (DCA).

**Results.** There was a statistically significant difference in the prevalence of CAE and BM patients in age ( $P < 0.001$ ), and there was no significant difference in sex ( $P = 0.539$ ). The difference between the training and test sets for all the DKI and ADL parameters was not statistically significant (all  $P > 0.05$ ). After univariate and multivariate logistic regression analysis, it was found that nDmean and nCBF in the parenchyma of the lesion and nKmean and nDmean in the edematous area were independent predictive factors for distinguishing CAE from BM. The nomogram was constructed based on the four independent predictors. The area under the ROC curve (AUC), cut-off value, sensitivity, and specificity of the training and validation sets were 0.942 and 0.989, 0.827 and 0.950, 87.2 and 100.0, 95.5 and 95.0, respectively. The calibration curves showed good agreement between the actual and predicted probabilities of the samples. The DCA shows that the model has high clinical value.

**Conclusion.** The nomogram based on DKI and ASL functional imaging has very good efficacy in identifying CAE and BM, which can be visualized, accurate and non-invasive to identify and provide guidance for the clinical decision.

## STUDY ON THE GROWTH ACTIVITY OF HEPATIC ALVEOLAR ECHINOCOCCOSIS BY MULTIMODAL MRI COMBINED WITH SERUM CIRCULATING FREE DNA

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**Objective.** Hepatic alveolar echinococcosis (HAE) grows aggressively in the liver, and its growth characteristics are important for diagnosis and treatment. In this study, the biological activity of hepatic alveolar echinococcosis was studied by multi-mode MR imaging combined with circulating free DNA (cfDNA) in serum.

**Methods.** Seventy-two patients with HAE diagnosed clinically in the Qinghai University Affiliated Hospital from August 2018 to January 2019 were included in the study. Serum cfDNA was detected before MRI. The scanning sequence includes MRI plain scan and dynamic contrast examination, DWI and MRCP water imaging sequence; MRI evaluates the location, size and imaging type of lesions. The evaluation indexes of activity include the range of high signal zone of DWI images (marginal zone), small vesicles and enhancement characteristics. The marginal zone, small vesicles and ADC values were measured by T2WI, MRCP and DWI sequences ( $b = 800 \text{ mm/s}$ ) to further evaluate the biological activity of the lesions. The correlation between the size, imaging type of lesions and serum cfDNA was analyzed.

#### Results:

1. Lesions characteristics: there were 92 lesions in 72 patients with HAE, including 16 lesions  $< 5 \text{ cm}$ , 36 lesions  $> 5\text{--}10 \text{ cm}$  and 40 lesions  $> 10 \text{ cm}$ . Types of lesions: solid type 51 cases, liquefied type 7 cases, multiple nodular type 4 cases, mixed type 4 cases and calcified type 6 cases.

2. Evaluation of lesion activity: 49 lesions (53.3%) showed mild annular enhancement in portal vein phase after dynamic contrast-enhanced scanning; On DWI images, 87 lesions had high signal (marginal zone) with limited diffusion, while 5 calcified lesions had no obvious marginal zone. The width of the marginal zone is between  $(0.28 \sim 0.98) \text{ cm}$ ; The average ADC value is about  $(1.10 \pm 0.17) \times 10^{-3} \text{ mm}^2/\text{s}$  measured at  $0.5 \text{ cm}$  in the marginal zone. Average ADC value of small vesicles is about  $(0.79 \pm 0.16) \times 10^{-3} \text{ mm}^2/\text{s}$ ; Compared with ADC, the difference was statistically significant ( $P < 0.05$ ).

3. Correlation between the size and imaging type of lesions and cfDNA: The cfDNA range of lesions with HAE  $\geq 10 \text{ cm}$  is  $(98 \sim 11770) \text{ ng/ml}$ , which is higher than that of small lesions  $(0 \sim 98 \text{ ng/ml})$ , and the correlation between them is statistically different ( $r=0.482$ ,  $P < 0.001$ ); cfDNA was found in all types, with calcified DNA being the lowest  $(0 \sim 2 \text{ ng/ml})$  and with solid DNA being the highest  $(0 \sim 11770 \text{ ng/ml})$ .

**Conclusion:** cfDNA is related to the size, imaging type and activity of HAE lesions, and MRI combined with cfDNA can better evaluate the biological activity of HAE lesions.

## CLINICAL APPLICATION OF ARTIFICIAL INTELLIGENCE IN THE ULTRASOUND CLASSIFICATION OF HEPATIC CYSTIC ECHINOCOCCOSIS

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**Relevance.** Hepatic cystic echinococcosis (HCE) is a zoonotic disease that occurs when the larvae of *Echinococcus granulosus* parasitize the livers of humans and mammals. HCE has five subtypes, and accurate subtype classification is critical for the choice of treatment strategy. To examine the clinical utility of artificial intelligence (AI) based on convolutional neural networks (CNNs) in the classification of HCE subtypes with ultrasound imaging.

**Methods.** Ultrasound images from 4,012 HCE patients at the First Affiliated Hospital of Xinjiang Medical University were collected between 2008 and 2020; 1,820 HCE images from 967 patients



were used as the training and validation sets for the construction of the AI model, and the remaining 6,808 images from 3,045 patients were used as the test set to evaluate the performance of the AI models. The 6,808 images were randomly divided into six groups and each group contained equal proportions of the five subtypes. Each group was analyzed by a resident physician. The accuracy of HCE subtype classification by the AI model and by manual inspection was compared.

**Results.** The AI HCE classification model showed good performance in the diagnosis of subtypes CE1, CE2, CE4, and CE5. The overall accuracy of the AI classification (90.4%) was significantly higher than that of the manual classification by physicians (86.1%;  $P < 0.05$ ).

**Conclusion.** CNN can better identify the five subtypes of HCE on ultrasound images, and should help doctors with little experience to more accurately diagnose HCE.

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## PREDICTING EXTRAHEPATIC METASTASES ASSOCIATED WITH HEPATIC ALVEOLAR ECHINOCOCCOSIS: AN MRI-BASED RADIOMICS MODEL

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**Relevance.** To develop and validate the MRI radiomics model for predicting extrahepatic multiple organ metastasis of hepatic alveolar echinococcosis, and to explore its predictive value for extrahepatic multiple organ metastasis in patients with hepatic alveolar echinococcosis.

**Materials and Methods.** 117 patients with hepatic alveolar echinococcosis were divided into a training ( $n = 83$ ) and test ( $n = 34$ ) group. Radiomic features were extracted from both the recovered T2-weighted/spectral adiabatic inversion and contrast-enhanced T1-weighted MRI images. The univariate rank sum test, correlation analysis, and multivariate stepwise regression techniques retained those features closely associated with extrahepatic metastases caused by hepatic alveolar echinococcosis. To develop and validate radiomics, clinical, and combined clinical Radiomics models for predicting extrahepatic metastases in hepatic alveolar echinococcosis.

**Results.** Six radiomic features were significantly correlated with hepatic alveolar echinococcosis-associated extrahepatic metastases. Compared with the T2-weighted model, the contrast-enhanced T1-weighted model had a better predictive performance. Hepatic vein involvement and CA125 levels were useful clinical predictors of hepatic alveolar echinococcosis-associated extrahepatic metastases. The nomogram that included radiomic signatures and clinical risk factors performed well, with an area under the curve, accuracy, and sensitivity of 0.861, 0.819, and 0.898 in the training cohort and 0.868, 0.765, and 0.950 in the test cohort respectively.

**Conclusion.** The contrast-enhanced T1-weighted radiomics model accurately predicted hepatic alveolar echinococcosis-associated extrahepatic metastases. A nomogram of the combined radiomic signature and clinical risk factors model provides an intuitive and reliable reference for the individualized evaluation of these patients.

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## EXPLORING THE VALUE OF REMOTE IMAGING AND MULTI-DISCIPLINARY TEAM IN DIAGNOSIS AND TREATMENT OF HEPATIC ECHINOCOCCOSIS IN QINGHAI PROVINCE

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**Relevance.** To explore the value of tele-imaging combined with a multidisciplinary collaborative team (MDT) in diagnosing and managing hepatic echinococcosis in Qinghai Province.

**Materials and Methods:** a retrospective analysis was performed of 100 hepatic echinococcosis patients anticipating surgery, of them the clinical images and information were transferred from primary hospital to Xikang group consultation platform of the imaging center in Qinghai University Affiliated Hospital from April 2019 to February 2020. The clinical and imaging data were collected to analyze the classification and staging of hepatic echinococcosis and the changes in diagnosis and treatment plan after MDT.

**Results.** The analysis showed that after remote imaging and MDT, of the 100 hepatic echinococcosis cases, one case of mixed echinococcosis was referred to superior hospital for surgical treatment; of 44 hepatic cystic echinococcosis patients, 34 were treated by surgery in local hospitals, 6 were referred to superior hospitals for surgical treatment, and 4 were treated with medication; of 55 hepatic alveolar echinococcosis cases, 30 patients received surgery locally, 10 patients were referred to the upper hospital for surgery, and 15 patients received drug treatment. After remote imaging consultation, the cyst classification at initial screening and the diagnosis and treatment scheme were adjusted for 48 cases.

**Conclusions.** It was demonstrated that remote imaging and MDT have great potential in improving the diagnosis and treatment of hepatic echinococcosis in primary hospitals in pastoral areas of Qinghai Province, providing a basis for the successful development of localized surgery and zero-clearance of surgical tech echinococcosis cases in Qinghai area, and saving the medical resources and costs as well.

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## EVALUATION OF A WESTERN BLOT ASSOCIATED WITH A LINE BLOT ASSAY FOR THE SERODIAGNOSIS OF ALVEOLAR AND CYSTIC ECHINOCOCCOSIS

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**Introduction.** Alveolar (AE) and cystic echinococcosis (CE), caused respectively by *Echinococcus multilocularis* (*E.m*) and *Echinococcus granulosus complex* (*E.g*), are severe parasitic zoonosis. Serology is currently used as a screening test and combines different methods including immunoblots which are highly specific and used for both confirmation and species differentiation. The Belgian National Reference Laboratory for Echinococcosis (BNRLE) has evaluated the anti-*Echinococcus* Euroline Western Blot (WB) (IgG) assay which uses *E.m* vesicle antigens, associated with a line blot, including recombinant antigens from *E.g* (EgAgB) and *E.m* (Em18 and Em95). This assay was compared with the LDBio WB assay which targets only *E.m* vesicle antigens and which is the current test used in the BNRLE.

**Material and Methods.** A total of 61 sera were retrospectively included. They were taken from 22 patients with CE, 23 with AE, 2 with toxocariasis and 14 negative controls. Two immunoassays were compared: the anti-*Echinococcus* Euroline WB (IgG) assay (Euroimmun, Germany) and the *Echinococcus* Western Blot IgG assay (LDBio Diagnostics, France). Unlike the LDBio assay based on a simple visual assessment of the bands, the interpretation of the Euroimmun assay is automated using the EUROLIneScan software (Euroimmun, Germany) which identifies the bands and measures their intensity. The McNemar test was used for statistical analysis.

**Results.** Among the 45 EA + EK cases, the identification at least to the genus *Echinococcus* was assigned to 38/45 (84%) and 44/45 (98%) of the patients by the Euroimmun and LDBio assays, respectively. Regarding species differentiation, correct identification to *E.m* was assigned to 48% (11/23) of AE cases with the Euroimmun assay versus 57% (13/23) with the LDBio assay. Of all the AE cases, two cross-reactions (2/23) with *E.g* were highlighted with the Euroimmun assay and none with the LDBio assay. Of the 22 CE cases, the Euroimmun and LDBio assays confirmed the presence of *Eg* for 64% (14/22) and 59% (13/22), respectively. Only one cross-reaction with *E.m* was highlighted with the Euroimmun assay and none with the LDBio assay. With regard to toxocariasis cases and negative controls, no cross-reaction was detected with the two assays. Statistical analysis demonstrates that there is no difference between the two assays for specificities and sensitivities (p-

value  $> 0,05$ ). However, the performance of the Euroimmun assay can still be improved by reducing the cut-off to an intensity of 10. By applying this new cut-off, we detected 6/7 of the false negative results for the genus *Echinococcus* and avoided 2/3 cross-reactions.

**Conclusion.** Overall, the Euroimmun assay showed slightly lower performance than the LDBio assay, although statistically there is no difference. However, when the cut-off is decreased, performance is similar or better than the LDBio assay. Besides, the Euroimmun assay offers many advantages such faster turn-around-time and standardized analysis by the EUROLIneScan software. Therefore, the Euroimmune assay is an effective serological method for the identification and species differentiation but the cut-off needs to be reassessed to further improve sensitivity and specificity.

## ANALYSIS OF CONTRAST-ENHANCED ULTRASOUND PARAMETERS IN DIFFERENT CLASSIFICATION OF HEPATIC ALVEOLAR ECHINOCOCCOSIS

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**Relevance.** To investigate the characteristics of quantitative parameters of contrast-enhanced ultrasound (CEUS) in different types of hepatic alveolar echinococcosis and its clinical value.

**Materials and methods.** Contrast-enhanced ultrasound was performed on 90 lesions of 57 patients with hepatic alveolar echinococcosis. The time-intensity curve (TIC) of contrast-enhanced ultrasound was drawn on the peripheral enhancement area of the lesion and the normal liver parenchyma, and the quantitative parameters of CEUS were compared between the two groups: Time to peak (TtoPK), peak intensity (PI), Area under time-intensity curve (AUC), and gradient of curve (Grad) were compared between different classification of HAE, and statistical analysis was performed.

**Results.** PI and AUC of the peripheral enhancement area of HAE lesions were smaller than those of normal liver parenchyma, and the differences between the two groups were statistically significant ( $t=-2.171, -2.859$ , both  $P < 0.05$ ). TtoP and Grad did not show significant difference ( $t=-0.416, -0.398$ ,  $P > 0.05$ ). There were significant differences in PI between different types of HAE ( $F=4.074, P < 0.05$ ). There were no significant differences in TtoP, AUC and Grad between different classifications of HAE ( $H=1.191, 1.549, F=0.052, P > 0.05$ ).

**Conclusion.** CEUS can clearly show the biological boundary of HAE and the characteristics of blood perfusion at the edge of HAE. The quantitative parameters of CEUS are different in different classifications of HAE, indicating that the lesions are in different growth stages and their growth activity is different, which provides a basis for clinical development of individualized diagnosis and treatment plan.

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# MOLECULAR DETECTION OF *ECHINOCOCCUS GRANULOSUS* EGGS FROM DOGS AND ENVIRONMENTAL SAMPLES IN SOUTH WEST NIGERIA

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Cystic echinococcosis (CE) is highly endemic in Nigeria, but to date the picture of the epidemiological situation for *Echinococcus granulosus sensu lato* (*s.l.*) in this area is still incomplete. Particularly in South West Nigeria, a limited amount of data on dogs' prevalence and environmental faecal contamination is available. To fill this gap, we focused on urban and semi-urban areas of the two major cities in South West Nigeria, Lagos (Lagos State) and Ibadan (Oyo State). We determined the prevalence of *E. granulosus s.l.* infection among owned dogs in Lagos (1) and we detected the presence of *E. granulosus s.l.* eggs in environmental samples in the city of Ibadan (2). The factors associated with *E. granulosus s.l.* infection among owned dogs and those with *E. granulosus s.l.* eggs dispersion were also evaluated. Faecal samples from owned dogs (n = 217) collected at veterinary clinics or hospitals in Lagos and soil (n = 200), faecal (n = 200) and water samples (n = 50) collected from the environment in Ibadan were examined by microscopic observation for the presence of taeniid eggs. The taeniid eggs were subsequently identified at molecular level using a multiplex PCR (3). Taeniid eggs were microscopically found in 6.0% of owned dog faecal samples from Lagos. PCR analyses confirmed the presence of *E. granulosus s.l.* infection among owned dogs in Lagos State with an overall prevalence of 5.5%. Based on the questionnaire administered to the dogs' owners it could also be evidenced that the location of the veterinary clinics or hospital and the purpose for keeping dogs were significant factors associated with *E. granulosus s.l.* infection. Dogs living in suburban areas and kept for security purposes were more likely to become infected.

Microscopic observation of the environmental samples from the city of Ibadan evidenced the presence of taeniid eggs in soil (11.5%), faecal (25.5%) and water samples (8.0%). *E. granulosus s.l.* was molecularly identified in 8.0%, 24.0% and 2.0% of soil, faecal and water samples, respectively. High levels of environmental contamination with *E. granulosus s.l.* eggs were found in urban and semi-urban areas of the city of Ibadan. The proximity to slaughterhouses, the level of urbanisation and the local government area of belonging did not seem to affect *E. granulosus s.l.* eggs dissemination patterns. The high prevalence in owned dogs and the extent of the environmental contamination found in South West Nigeria raises public health issues and suggests the adoption of appropriate measures to reduce risk of *E. granulosus s.l.* transmission to humans. Appropriate and regular treatment based on the recommendations of a veterinarian is highly recommended in owned dogs. The prevention strategies against CE should also consider the control of stray dogs, the establishment of deworming programs and the promotion of public education.

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## INVESTIGATION AND GENETIC POLYMORPHISM ANALYSIS OF RODENTS INFECTION WITH *ECHINOCOCCUS* IN ILI REGION, XINJIANG

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**Relevance.** Animal husbandry in Ili region of Xinjiang is well developed, the number of dogs is large, the pasture is abundant, and the rodent infestation is serious. Recent investigations have revealed the presence of wild red foxes in the region, and domestic animals-canines/foxes-rodents constitute the cycle chain of echinococcosis, which also facilitates the spread of echinococcosis. However, there are relatively few reports on *Echinococcus* infection and *Echinococcus* gene polymorphism in rodents in this area.

**Materials and methods.** To understand the murine *Echinococcus* infection, distribution, and pathogenic gene polymorphisms in Ili, Xinjiang, the collected rodent samples were identified according to their morphological characteristics and skull morphology. Histopathological examination of *Echinococcus* carried by rodent was performed. Meanwhile, PCR amplified the mitochondrial *nad1* gene of the pathogen, carried out phylogenetic analysis, haplotype analysis and haplotype network map construction, and statistical analysis the infection of *Echinococcus* in different regions and different rodent species.

**Results.** The captured rodents belonged to 3 species by morphological identification. Tissue sections of liver lesions showed protoscoleces. A band of about 510 bp was amplified by PCR from 42 samples, and sequence alignment showed that all of them were *Echinococcus multilocularis*. The homology with the gene sequences of *Echinococcus multilocularis* (GenBank login numbers: MN444805, MH259778, AJ237639, EU704122) was 98%~100% and clustered into one branch in the phylogenetic tree. The results of haplotype analysis showed that there were 21 haplotypes in the 42 sequences, of which Hap2 was the main haplotype. Statistical analysis shows that the total infection rate of *Echinococcus multilocularis* in rodents in different regions was 15.73% (42/267), and there was no significant difference in infection rates among different regions ( $\chi^2=5.119$ ,  $P>0.05$ ) and different rodants species ( $\chi^2=1.364$ ,  $P>0.05$ ). Compared with the survey results of Guo in 2017, the AE infection in Ili area has an increasing trend, which may be due to the differences in sampling areas.

**Conclusion.** The pathogen of *Echinococcus* infection in rodent in Ili area is *Echinococcus multilocularis*, and Hap2 is the main haplotype for *Echinococcus multilocularis nad1* gene in this area.

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DIFFERENCES IN CYST CHARACTERISTICS IN HUMANS CAUSED BY  
*ECHINOCOCCUS GRANULOSUS SENSU STRICTO* AND THE G6 GENOTYPE OF *E.*  
*GRANULOSUS SENSU LATO*

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**Relevance.** Differences in cyst development [1] and organ tropism [2] in humans have been suggested between the infection with *E. granulosus s.s.* and the G6 genotype. However, limited data exists confirming differences in clinical manifestations of CE [3].

**Materials and methods.** In this study, 124 *Echinococcus* cysts acquired from 90 CE between 2014 and 2018 were studied. Patient information, including origin, age, gender, infected organ/tissue, cyst diameter, and the number of cysts, was documented.

**Results.** *E. granulosus s.s.* was identified in 51 individuals (56.7%), comprising 81 cysts, while the G6 genotype was found in 39 patients (43.3%), with 43 cysts. *E. granulosus s.s.* was more prevalent in the liver (32/51 patients), whereas the G6 genotype was associated with lung and extrahepatic localizations (27/39 patients). Patients infected with *E. granulosus s.s.* presented up to 6 cysts, whereas those infected with G6 displayed a maximum of 2 cysts. *E. granulosus s.s.* lung cysts were larger than G6 cysts.

**Conclusion.** This study offers evidence of clinical differences in cystic echinococcosis caused by *E. granulosus s.s.* and the G6 genotype of the *E. granulosus sensu lato* complex in humans. The implications for treatment and control are also discussed.

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UNEXPECTED ECHINOCOCCUS CANADENSIS  
(GENOTYPE G7) DETECTED IN CYPRUS

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Cystic echinococcosis (CE), caused by the dog tapeworm *Echinococcus granulosus sensu lato*, represented a major public health issue in Cyprus during the last century until a first island-based control programme implemented from 1971 to 1985, seemed to have succeed in eradicating the disease [1]. However, it was soon demonstrated that *E. granulosus s.l.* was still circulating in the island, although at lower prevalences, therefore leading to the reintroduction of control programmes [2].

A coproantigen ELISA prevalence in dogs up to 3.58%, represents the most recent estimate of the epidemiological situation in Cyprus [3]. No prevalence data about humans or wildlife has been available after the 2000's, and no genotyping data has been achieved so far, with the exception of a specimen of *E. granulosus sensu stricto* isolated in a mouflon (A. Casulli, *pers comm*).

On February 2023, *Echinococcus granulosus s.l.* adult worms were detected during the microscopic examination of the intestinal mucosa of a stray dog coming from the Government-controlled area of Cyprus by sedimentation and counting technique (SCT). Two worms were preserved in ethanol 70% and referred to the European Reference Laboratory for Parasites (EURLP) at the Istituto Superiore di Sanità (Rome, Italy) for molecular analyses. A PCR-RFLP and Multiplex PCR method [4] identified the worms as *Echinococcus canadensis*, cluster G6/G7. Subsequently, the sequence analysis of the nad2 and nad5 mitochondrial genes allowed to assign the two samples to the G7 genotype and more specifically to the haplogroup designated as G7b, previously identified in the islands of Corsica (France) and Sardinia (Italy) [5]. This finding represents the first molecular characterization of *E. canadensis* in Cyprus, to the authors' knowledge. It would be relevant to analyze more samples, from domestic and wild animal species, as well as human samples, to elucidate the recent transmission dynamics of *Echinococcus* species occurring in the island.

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## CYSTIC ECHINOCOCCUS IN PAKISTAN: A COUNTRY MANIFESTING DECADES OF NEGLECT

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**Relevance.** Cystic echinococcosis (CE) is a significant zoonotic disease globally [1] and is ranked as 4th most widespread helminth disease in Pakistan [2]. The current status of CE remains unclear due to lack of large scale surveillance studies. Designing a comprehensive management and control strategy requires assessment of disease epidemiology, key endemic zones and risk factors to understand transmission dynamics [3].

**Materials & methods.** The study was based on exploration of main genotypes involved in implication of CE in animals and humans based partial mitochondrial *nad5* gene. A retrospective study was conducted to determine the frequency of human CE in five cities of Punjab province. Clinical records of infected patients (n = 86) from hospitals were retrieved from 2008-2022. A total of 35 hydatid cyst specimens were also collected from these hospitals. Additionally, 29 cysts were collected from the sheep (n = 8), cattle (n = 11) and buffaloes (n = 10) from Multan, Bahawalpur and Sargodha. The computation of different genetic and population diversity indices such as number of haplotypes, nucleotide diversity, haplotype diversity, Tajima's *D*, Fu's *F<sub>s</sub>* and *F<sub>st</sub>* was carried out for the obtained nucleotide sequences from humans and domestic ruminants.

**Results.** Only 10 human samples were successfully amplified which yielded two genotypes of *Echinococcus granulosus* [G1 (n = 3) and G3 (n = 7)]. Likewise, four genotypes [(G1 (n = 4), G3 (n = 20), G5 (n = 4), G6 (n = 1))] were identified from the animals. The G3 genotype had predominantly higher percentage (>60%) among both humans and animals. A high value of haplotype diversity ( $0.812 \pm 0.063$ ) coupled with low nucleotide diversity ( $0.00432 \pm 0.0071$ ) was observed. Population demographics and genetic variability indices suggested expanding parasitic population.

**Conclusion.** It was demonstrated that the G3 genotype was predominantly implicated in human and animal CE as opposed to other regions of the world where globally G1 strain is usually prevalent. For a middle-income country like Pakistan where basic health facilities are scarce, CE remains fairly under-investigated and thus neglected in comparison to other morbidities. Poor knowledge and lack of consideration for CE leads to challenges like misdiagnosis and mistreatment. In cases where data is accessible, misreporting is often observed due to negligent attitude of healthcare staff about NTDs [4]. CE is particularly prevalent in marginalized and rural communities [5] which are frequently overlooked by the public health department.

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## IDENTIFICATION OF *ECHINOCOCCUS GRANULOSUS SENSU LATO* FROM SHEPHERD DOGS IN SARDINIA, ITALY (MeME project)

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**Background.** *Echinococcus granulosus sensu lato* (*s.l.*) is the aetiological agent of cystic echinococcosis (CE), a neglected zoonotic disease that remains a major concern for human health. Five different species of *E. granulosus s.l.* have been recognized to date: *E. granulosus sensu stricto* (*s.s.*), *E. equinus*, *E. ortleppi*, *E. canadensis* and *E. felidis*. The dog is the main definitive host of *E. granulosus s.l.* responsible for transmission of the parasite to livestock (mainly sheep) and accidentally to humans. This study, as part of the MeME project (One Health EJP; GA 773830), aimed to detect the presence of four species belonging to *E. granulosus s.l.* in shepherd dogs in Sardinia (Italy).

**Material and Methods.** For this purpose, 203 dog fecal samples were collected from 90 sheep farms. DNA samples extracted from dog faeces were analysed using four real-time PCR assays in order to detect and identify species within *E. granulosus s.l.* (*E. granulosus s.s.*, *E. equinus*, *E. ortleppi* and *E. canadensis*).

**Results.** Our results showed that 18 samples were positive for *E. granulosus s.s.*; no other *E. granulosus s.l.* species were identified. In Sardinia, a high prevalence of *E. granulosus s.s.* infection (8.9%) in shepherd dogs, living in close contact with sheep, was found.

**Conclusion.** Identification to the species level within *E. granulosus s.l.* is necessary to identify the hosts involved and thus to implement more effective strategies for the prevention and control of CE. Further studies are suggested to define the complete epidemiological scenario of CE in endemic areas.



THE GENETIC DIVERSITY OF ECHINOCOCCUS MULTILOCULARIS  
IN EUROPE: A PICTURE BASED ON MITOCHONDRIAL MARKERS

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The cestode *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis, a highly fatal zoonotic parasitic disease of the northern hemisphere [1]. Red foxes are the main reservoir hosts and, probably, the main drivers of the geographic spreading of the disease in Europe. Knowledge of genetic relationship among *E. multilocularis* isolates at European scale is a key to understand the dispersal characteristics of *E. multilocularis* [2,3]. Hence, the present study aimed to describe the genetic diversity of *E. multilocularis* isolates obtained from different hosts in 19 European countries. Based on the analysis of complete nucleotide sequences of the *cob*, *atp6*, *nad2*, *nad1* and *cox1* mitochondrial genes (4,968 bp), 43 haplotypes were inferred. Four haplotypes represented 62.56% of the examined isolates (142/227), and one of these four haplotypes was found in each country investigated, except Svalbard (Norway). While the haplotypes from Svalbard were markedly different from all the others, mainland Europe appeared to be dominated by two main clusters, represented by most western-central-eastern European countries, and the Baltic countries including North-East Poland, respectively. Further comparison was made with the *cob*, *nad2* and *cox1* Nakao's historical haplotypes [4], unveiling the presence of one Asian-like haplotype identified in Latvia and North-Eastern Poland. To better elucidate the presence of Asian genetic variants of *E. multilocularis* in Europe, and to get a more comprehensive European wide coverage, further studies, including endemic regions not investigated in the present study, especially some Eastern European countries, are needed.

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## **GENETICS OF *ECHINOCOCCUS* – PAST, PRESENT, FUTURE. LESSONS LEARNED AND FUTURE PERSPECTIVES**

**Teivi Laurimäe**

A vast number of studies exploring the genetic variability of the genus *Echinococcus* have been published in the last decades, with the majority of these focusing on either *E. multilocularis* or on the species belonging to the group that is collectively referred to as *E. granulosus sensu lato* (s.l.). These studies have predominantly involved the use of mitochondrial (mt) gene fragments, while in recent

years complete mt genes and even complete mt genomes have been utilized as well. So far, the extent of genetic variability of the nuclear genomes of these parasites has not been extensively investigated. As the Next Generation Sequencing (NGS) technology becomes more accessible and high-quality reference genome(s) are made available, the possible information that could be produced by genome-wide explorations could ultimately lead to a better understanding of the impact of nuclear genomic variation on the biology of these parasites. Here we aim to give an overview of the current state-of-the-art knowledge on the genetics of the genus *Echinococcus*, mainly focusing on the species having the highest economic and public health importance, namely *E. granulosus* s.l. and *E. multilocularis*, and discuss possible implications and future perspectives.

### **DISTRIBUTION AND GENETIC DIVERSITY OF CYSTIC ECHINOCOCCOSIS IN WESTERN KENYA**

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**Relevance.** This study determined the presence of CE in humans, the genetic variability of the parasite in livestock and the role of dogs in the establishment of lifecycle in a non-endemic region.

**Materials and Methods.** Screening of human CE in Bungoma County was performed using a portable ultrasound scanner, Copro-ELISA was performed on the dog faecal samples, sequencing performed to genotype taeniid eggs, characterize CE livestock cysts and determine the genetic diversity of *E. granulosus* sensu stricto (s. s.).

**Results.** Out of 1002 people screened for CE; 67 (6.7%) participants had abnormal findings and, of these, 7 (1.1%) had simple liver cysts classified as CL by WHO. In livestock, *E. granulosus* s. s. was identified in 135/153 cysts. Eleven haplotypes for *Nad1* and 19 for *Cox1* gene were identified in *E. granulosus* s. s. The sequences of nine (9) taeniid eggs recovered from the single taeniid positive sample Copro-ELISA was positive in 12/77 (15.6%) faecal samples.

**Conclusion.** This study report for the first time *E. granulosus* s. s. haplotypes in livestock in east Africa and the establishment of *E. granulosus* s. l. and taeniids life cycle in a non-endemic region

### **MOLECULAR DISCRIMINATION OF G1 AND G3 GENOTYPES OF ECHINOCOCCUS GRANULOSUS SENSU STRICTO ISOLATES OBTAINED FROM HUMAN, CATTLE AND SHEEP IN TURKEY BY USING OF THE MITOCHONDRIAL NAD5 MARKER**

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Cystic echinococcosis (CE), caused by *Echinococcus granulosus* sensu lato (sl), is a zoonotic disease prevalent worldwide. The worm has an indirect biology that includes final and intermediate hosts. Egg propagation is made by adult forms located in the small intestines of dogs and wild

carnivores, which are their final hosts. In intermediate hosts, which are generally herbivores, larval forms develop in their internal organs by oral intake of the worm's eggs. Humans are accidentally infected and ingesting the eggs causes CE. This study was carried out to determine the genotype and haplotype differences by using of *nad5* gene in hydatid cyst samples which were confirmed to be *E. granulosus s.s.* Human (n=12), cattle (n=28) and sheep (n=31) hydatid cyst isolates were included in the study. A total of 71 gDNA samples, including 12 human, 28 cattle and 31 sheep samples, were successfully extracted and 759 bp long mt-*nad5* gene fragment was amplified by PCR. Following the sequence analysis, *E. granulosus s.s.* was defined as G1 (n=61) and G3 (n=10) genotypes. A total of 23 haplotypes were obtained from 71 *E. granulosus s.s.* G1 and G3 samples. The main haplotype was Hap01 (60.56%), which consisted of G1 genotype. The second largest haplotype was Hap04, which consisted entirely of G3 genotype. Hap14 acts as a bridge between G1 and G3 genotypes. With the present study, it was revealed that the G1 strain of *E. granulosus s.s.* is the dominant genotype in humans and farm animals in Turkey, as in other countries. However, the high haplotype and nucleotide diversity in genotypes was remarkable. Therefore, in countries such as Turkey, where CE is common in humans and animals, regular monitoring of genetic variations in *E. granulosus s.s.* is important in terms of phylogeographic progression.

### CYSTIC ECHINOCOCCOSIS IN PAKISTAN: GENOTYPING OF ECHINOCOCCUS GRANULOSUS SENSU STRICTO IN HUMAN CYST ISOLATES

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**Background.** Cystic Echinococcosis (CE) is a zoonotic disease with a worldwide diffusion, representing an important public health issue in South Asia (Khan et al., 2020). The larval form of *Echinococcus granulosus sensu lato* (*s.l.*) is the etiologic agent of CE. Within the *E. granulosus s.l.* complex *E. granulosus sensu stricto* (*s.s.*) is responsible of the vast majority of human CE cases (1). To the best of our knowledge, few molecular studies on *E. granulosus* have been currently performed in Pakistan.

The present study aimed to molecularly characterize CE cysts from human patients from different hospitals in Khyber Pakhtunkhwa, Pakistan.

**Material and Methods.** A total of 251 Formalin-Fixed Paraffin-Embedded (FFPE) tissues from human CE cysts, sampled from 2006 to 2021, were collected from four major hospitals of Peshawar (Khyber Pakhtunkhwa, Pakistan) comprehensive of 50 Afghan immigrants. The majority of patients belongs to age group 16-35 (40.5%, 42.5%) followed by 36-55 (27.8%, 27.4%).

A total of 178 genomic DNAs (gDNAs) were successfully extracted from FFPE tissues using a commercial kit, QIAamp DSP DNA FFPE Tissue (Qiagen, Hilden, Germany). The DNA samples were analyzed using a Taq Man Real Time PCR assay for *E. granulosus s.s.* identification. Positive samples were further examined by a SNP genotyping assay (3) for G1 and G3 genotype discrimination.

**Results.** Out of 178 gDNAs, 146 (82.02%) positive results for *E. granulosus s.s.* were found. Unfortunately, DNA extraction from FFPE samples provided the required DNA yield and quality in only 104 *E. granulosus s.s.* positive samples, allowing the identification of 82 G1 and 22 G3 genotype.

Majority of patients presented hepatic CE (36.9%) followed by spleen (4.10%) and lungs (3.17%). Highest frequency was recorded in females (50.7%) respect to males (42.9%). Conversely, among the 50 (28.1%) Afghan immigrants, males and females were equally infected (50%) being the highest cases



in liver (36%) followed by spleen and subtotal thyroidectomy (4%) and some unique sites like neck, shoulder, peritoneal cavity, brain, kidney, abdomen, parietal and posterior fossa were also detected.

**Conclusion.** This study evidenced a high percentage of human CE positive cases from the Khyber Pakhtunkhwa province and from the Afghan immigrants in Pakistan. Molecular analysis demonstrated that it is mainly caused by *E. granulosus* s.s. genotype G1. However, hospital records are mostly partial since registries are not structured, and they do not report all CE cases managed. Thus, this study confirmed cystic echinococcosis as major health issue.

**References.** Khan et al., Cystic Echinococcosis in Pakistan: A Review of Reported Cases, Diagnosis, and Management. Acta Trop. 2020 Dec;212:105709.

## EVALUATION OF A WESTERN BLOT ASSAY ASSOCIATED WITH A LINE BLOT FOR THE SERODIAGNOSIS OF ALVEOLAR AND CYSTIC ECHINOCOCCOSIS

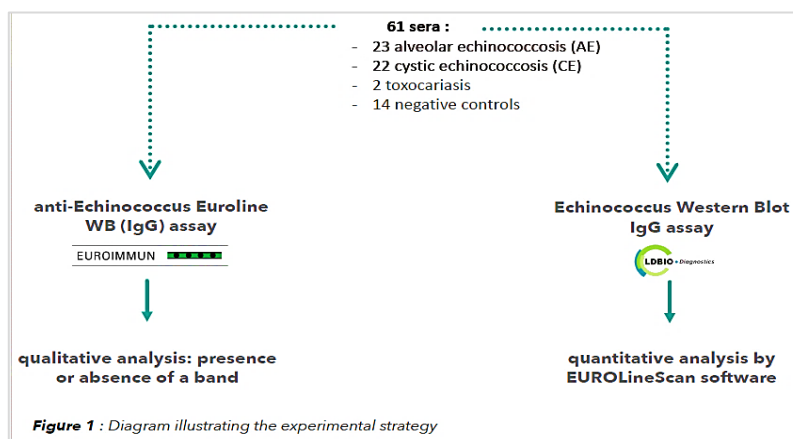
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**Introduction.** Alveolar (AE) and cystic echinococcosis (CE), caused respectively by *Echinococcus multilocularis* (E.m) and *Echinococcus granulosus complex* (E.g), are severe parasitic zoonosis. Serology is currently used as a screening test and combines different methods including immunoblots which are highly specific and used for both confirmation and species differentiation. The Belgian National Reference Laboratory for Echinococcosis (BNRLE) has evaluated the anti-*Echinococcus* Euroline Western Blot (WB) (IgG) assay which uses *E.m* vesicle antigens, associated with a line blot, including recombinant antigens from *E.g* (EgAgB) and *E.m* (Em18 and Em95). This assay was compared with the LDBio WB assay which targets only *E.m* vesicle antigens and which is the current test used in the BNRLE.

**Methods.** A total of 61 sera were retrospectively included. They were taken from 22 patients with CE, 23 with AE, 2 with toxocariasis and 14 negative controls. Two immunoassays were compared: the *anti-Echinococcus Euroline WB (IgG) assay* (Euroimmun, Germany) and the *Echinococcus Western Blot IgG assay* (LDBio Diagnostics, France). Unlike the LDBio assay based on a simple visual assessment of the bands, the interpretation of the Euroimmun assay is automated using the EUROLinScan software (Euroimmun, Germany) which identifies the bands and measures their intensity. The McNemar test was used for statistical analysis. According to the manufacturer, the set cut-off values are between an intensity of 13 and 17. **Figure 1** illustrates the experimental strategy of the study.





**Results.** Among the 45 EA + EK cases, the identification at least to the genus *Echinococcus* was assigned to 38/45 (84%) and 44/45 (98%) of the patients by the Euroimmun and LDBio assays, respectively. Regarding species differentiation, correct identification to *E.m* was assigned to 48% (11/23) of AE cases with the Euroimmun assay versus 57% (13/23) with the LDBio assay. Of all the AE cases, two cross-reactions (2/23) with *E.g* were highlighted with the Euroimmun assay and none with the LDBio assay. Of the 22 CE cases, the Euroimmun and LDBio assays confirmed the presence of *E.g* for 64% (14/22) and 59% (13/22), respectively. Only one cross-reaction with *E.m* was highlighted with the Euroimmun assay and none with the LDBio assay. With regard to toxocariasis cases and negative controls, no cross-reaction was detected with the two assays. Statistical analysis demonstrates that there is no difference between the two assays for specificities and sensitivities (p-value >0,05). However, the performance of the Euroimmun assay can still be improved by reducing the cut-off to an intensity of 10. By applying this new cut-off, we detected 6/7 of the false negative results for the genus *Echinococcus* and avoided 2/3 cross-reactions. Table 1 summarizes the sensitivities and specificities obtained with the two methods.

Table 1 - Sensitivities and specificities obtained for the Euroimmun and LDBio assays

	EUROIMMUN assay		LDBIO assay
	Cut-off values set by manufacturer (intensity between 13 and 17)	Decreased cut-off values (intensity between 10 and 17)	
<b>Sensitivity</b>	Sensitivity to <i>Echinococcus</i> genus (n=45)	84% (38/45)	98% (44/45)
	Sensitivity to <i>E.m</i> species (n=23)	48% (11/23)	57% (13/23)
	Sensitivity to <i>E.g</i> species (n=22)	64% (14/22)	59% (13/22)
<b>Specificity</b>	Specificity to <i>Echinococcus</i> genus (n=16)	100% (16/16)	100% (16/16)
	Specificity to <i>E.m</i> species (n=38)	97% (37/38)	100% (38/38)
	Specificity to <i>E.g</i> species (n=39)	95% (37/39)	100% (39/39)

### Conclusions:

- Overall, the Euroimmun assay showed slightly lower performance than the LDBio assay, although statistically there is no difference.
- When the cut-off is decreased, performance of Euroimmun assay is similar or better than the LDBio assay.
- Euroimmun assay offers many advantages such faster turn-around-time and standardized analysis by the EUROLInScan software.
- Euroimmun assay is an effective serological method for the identification and species differentiation but the cut-off needs to be reassessed to further improve sensitivity and specificity.

**РОЛЬ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА  
В РАЗВИТИИ ЭХИНОКОККОЗА**

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**Введение.** Кистозный эхинококкоз – заболевание, вызываемое личиночной стадией цестоды *Echinococcus granulosus*. Собаки выступают в качестве окончательных хозяев, тогда как травоядные и человек выступают в качестве промежуточных хозяев [1]. Несмотря на значительное совершенствование методов инструментальной диагностики эхинококкозов, повышение доступности высокотехнологичных видов медицинской помощи, данные ларвальные гельминтозы остаются актуальной междисциплинарной проблемой. Социальная их значимость обусловлена поражением лиц трудоспособного возраста, проживающих в сельской местности и высокими показателями летальности[2]. Кыргызстан остается неблагополучным гиперэндемичным регионом по распространенности эхинококкозов, имеющих статус общегосударственной проблемы ввиду широкого распространения и огромного экономического ущерба для здравоохранения[3]. Изучение резистентных барьерных механизмов желудочно-кишечного тракта, в частности роль желудка и ферментов поджелудочной железы в проникновении яиц эхинококка в организм человека обуславливает актуальность проведенного исследования.

**Цель работы:** определить степень участия барьерных механизмов желудочно-кишечного тракта в проникновении яиц эхинококка во внутреннюю среду организма и в развитии эхинококковой кисты.

**Материалы и методы:** метод флотации кала собаки для выделения яиц эхинококка, экспериментальный и гельминтоовоскопия с фотографией.

**Результаты.** Для изучения роли желудочно-кишечного тракта в развитии эхинококкоза и влияния последнего на степень разрушения структуры яиц эхинококка моделировали процесс (экспериментально – *in vitro*), прохождения яиц эхинококка через желудочно-кишечный тракт и возможность их разрушения желудочным соком (различной кислотности) и ферментами поджелудочной железы. Проведены 3 серии опытов.

На I серии эксперимента - моделировали процесс переваривания яиц эхинококка в желудке при нормальной, повышенной и пониженной кислотности желудочного сока и под действием фермента ацидин-пепсина. На II этапе провели моделирование процесса переваривания яиц эхинококка в 12 перстном кишечнике. На III серии эксперимента изучили действие панкреатина на яйца эхинококка.

**Обсуждение.** При сравнении структуры яйца эхинококка в физиологическом растворе и при действии имитируемого желудочного сока разной кислотности, а также под действием пепсина и панкреатина выявили разные структурные изменения яйца эхинококка.

**Выводы.** Пониженная кислотность желудочного сока не влияла на структуру оболочек яйца эхинококка. Желудочный сок нормальной кислотности (соляная кислота и пепсин) вызывают набухание оболочки и частичную перестройку структуры яиц эхинококка, что не исключает возможности их проникновения во внутреннюю среду организма. При повышенной кислотности желудочного сока, в сочетании с действием ферментов трипсина и амилазы (и, или самостоятельно) приводит к полной деструкции оболочек и внутренней структуры яиц эхинококка.

**Рекомендации.** Людей имеющих пониженную кислотность желудочного сока и нехваткой ферментов ЖКТ взять на учет с диспансерным наблюдением и провести профилактическое лечение.

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## CEREBRAL ALVEOLAR ECHINOCOCCOSIS INVADING SAGITTAL SINUS AND SKULL: A CASE REPORT

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**Objective.** There have been very few reports on multiple-organ AE, especially AE in skull and bones. We report a rare case of disseminated multiple-organ AE invading sagittal sinus and skull.

**Patient concerns.** Eight years ago, the patient was admitted because respiratory dyspnea. The CT and MRI scan showed multiple nodules and masses in bilateral lungs, the left lateral lobe of the liver, and left parietal lobe of the brain, liver-derived systemic multi-organ echinococcosis was highly suspected. The patient underwent "left liver resection" and was pathologically diagnosed as alveolar echinococcosis (P2N0M1 IV stage). Subsequently, the patient took albendazole tablets and liposomes intermittently. During the follow-up period, the patient's intracranial hydatid lesions continue to grow and expand, leading to recurrent epilepsy, headache, numbness of the right limb and weakness of the right limb. The patient found a mass under the scalp at the top of the skull. Finally, the patient decided to accept surgical treatment. Most of the lesions were surgically removed, only on both sides of the sagittal sinus, a thin layer of lesions remained to protect the sagittal sinus from being destroyed. The patient recovered smoothly and was transferred to the rehabilitation department one week later.

**Outcomes.** After one year of follow-up, no residual lesions were found to be enlarged. The muscle strength of the right upper limb and the right lower limb was grade 3 and 5-, so she could stand and walk independently. After operation, she continued to take albendazole tablets and sodium valproate, without headaches and seizures.

**Conclusion.** For AE invading skull and located in functional area, surgery should be more aggressive.

## TREATMENT OF CEREBRAL ECHINOCOCCOSIS IN CHILDREN BY POSTURAL METHOD COMBINED WITH DOWLING-ORLANDO TECHNIQUE

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**Objective.** To introduce a more efficient method to remove cystic echinococcosis in children.

**Methods.** The surgical data of all children with cerebral cystic echinococcosis who underwent surgery from 1985 to 2023 were analyzed. Surgical methods: In the operation of children's cystic echinococcosis, the position of head change was adopted to make the center of gravity of the hydatid cyst outside the incision margin of cerebral cortex, combined with a small amount of warm saline instilling between the cyst and surrounding brain to make it naturally come out;

**Results.** From 1985 to 2023, 39 cases of brain hydatid disease in children under 18 years old were operated in the first affiliated hospital of Xinjiang medical university. There were 24 males (61.54%) and 15 females (38.46%). 2-18 years old, with a median age of 10 years old; In addition to brain hydatid disease, 13 cases lesions involved heart, liver, lung and spine; The operation was performed by postural method combined with Dowling-Orlando technique; There was no perioperative death; Follow-up time was 1-10 years, with recurrence in 2 cases (5.13%) and death in 2 cases (5.13%).

**Conclusion:** postural method combined with Dowling-Orlando technique is a safe and effective surgical method, which reduces the amount of saline injection and is beneficial to brain protection.

**Key words:** Dowling-Orlando technique; Echinococcosis; Hydatid cyst; Hydrodissection; Intracerebral cyst; Neurohydatidosis; Surgical technique.

## OLD DRUG REPURPOSING FOR NEGLECTED DISEASE: PYRONARIDINE AS A PROMISING CANDIDATE FOR THE TREATMENT OF ECHINOCOCCUS GRANULOSUS INFECTIONS

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Drug repurposing strategy was employed to identify new therapeutic agents against cystic echinococcosis (CE). An in vitro protoscolicidal assay was applied in the drug screening to identify pyronaridine, an approved antimalarial drug, for the treatment of CE. Following a three-dose intraperitoneal regimen (57 mg/kg, q.d. for 3 days), pyronaridine caused 100% cyst mortality. Oral administration of pyronaridine at 57 mg/kg, q.d. for 30 days significantly reduced the parasitic burden in the pre-infected mice compared with albendazole group ( $p < 0.001$ ). Using a microinjection (a mimic of the clinical PAIR technique) of drug into cysts, pyronaridine (200  $\mu$ M) showed highly effective in term of inhibition of cyst growth ( $p < 0.05$ ). Pharmacokinetic analysis revealed that pyronaridine was highly distributed in the liver and lungs, the most affected organs of CE. Function analysis showed that pyronaridine inhibited the activity of topoisomerase I ( $IC_{50} = 209.7 \pm 1.1 \mu$ M), and classical apoptotic hallmarks, including DNA fragmentation and caspase activation, were triggered. Given its approved clinical safety, the repurposing of pyronaridine offers a rapidly translational option for treating CE including PAIR.

## SERUM RAMAN SPECTROSCOPY COMBINED WITH DEEP LEARNING ALGORITHM FOR RAPID SCREENING OF TWO TYPE HEPATIC ECHINOCOCCOSIS

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**Relevance.** Echinococcosis is a neglected zoonotic parasitic disease [1], and early screening for initial diagnosis of echinococcosis is particularly critical. The diagnosis of echinococcosis is based primarily on imaging complemented by serology and is not convenient for the screening and epidemiologic survey of echinococcosis [2]. In this work, we established and evaluated a simple and accurate detection technique for rapid detection of hepatic alveolar echinococcosis (AE) and hepatic cystic echinococcosis (CE) by combining label free Raman scattering microscopy with machine learning.

**Materials and methods.** Serum Raman spectra of 458 patients with liver cirrhosis, hepatocellular carcinoma (HCC), AE, CE and normal control (NC) were used to establish the model, and 115 serum samples were used to evaluate the model. A classification diagnosis model was established using Convolutional Neural Networks (CNN), Support vector machine (SVM), K-Nearest Neighbor (KNN), Decision Tree (DT), Random Forest (RF), and linear discriminant analysis (LDA) algorithms respectively, and the diagnostic effectiveness of the classification model was evaluated using sensitivity, specificity, and accuracy.

**Results.** Among the four categories (liver cirrhosis, HCC, echinococcosis, and NC), the accuracy (95%) of CNN algorithms is higher than that of traditional machine learning algorithms. Among the five categories (liver cirrhosis, HCC, AE, CE and NC), its classification accuracy was 89.5%. In the classification of echinococcosis and non-echinococcosis, the classification accuracy of CNN model (94.8%) was higher than that of Dot immunogold filtration assay (DIGFA) (87.7%). In the classification of non-echinococcosis, AE and CE, the accuracy of CNN (88.7%) was higher than that of DIGFA (84.3%).

**Conclusion.** Raman spectroscopy combined with CNN algorithm has potential to be applied in the screening and epidemiologic survey of echinococcosis.

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## EVOLUTION OF CLINICAL PRESENTATION, TREATMENT AND PROGNOSIS OF PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS TREATED AT THE UNIVERSITY HOSPITAL ZURICH: A 50-YEAR EXPERIENCE

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**Aim.** The study aim was to evaluate the evolution in the presentation, treatment and prognosis of AE patients over the past 50 years.

**Methods.** Retrospective cohort study of 332 AE patients who were treated at the University Hospital Zurich between 1973-2022. Analysis included patient demographics, symptomatology, AE stage according to PNM classification, treatment type, outcome and overall survival; stratified by decade of diagnosis.

**Results.** Over the decades patient demographics did not change significantly, with a median age at diagnosis of 58 years. Since 2000 a steady increase in new diagnosis of AE was observed, with an increasing proportion attributable to incidental diagnosis. This was accompanied by a shift towards earlier stages. The 15-year overall survival rate remained consistent at 80% throughout the decades without significant variation and association with the disease stage. Only very few AE-related fatalities were recorded. Median age at death was 77 years.

**Conclusion.** Significant changes in incidence, stage at presentation and treatment of AE patients were observed over the past 50 years. Impact of AE on overall survival was minimal.

## COMPARATIVE IN VITRO EFFECT OF SILVER NITRATE AND HYPERTONIC SODIUM CHLORIDE ON PROTOSCOLICES OF HYDATID CYST IN A SHORT PERIOD TIME

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A study was made in order to compare the protoscolicidal effects of silver nitrate 0.5% and hypertonic sodium chloride 20% in short periods up to 5 minutes. Cetrимide 0.5% with very high protoscolicidal effect as positive control and normal saline 0.9% as a negative control were also used in our tests. A total of 3000-4000 protoscolices with a viability of over 90% were separately exposed to 1 milliliter of cetrимide 0.5%, hypertonic sodium chloride 20%, silver nitrate 0.5% and normal saline at different time periods of 1, 2 and 5 minutes followed by their viability evaluation. At 5 minutes, there was no significant difference between cetrимide 0.5% and hypertonic sodium chloride 20% regarding their protoscolicidal effect ( $p=1$ ), while at this exposure time, the difference between cetrимide 0.5% and silver nitrate 0.5% was significant regarding their protoscolicidal effect ( $p=0$ ). Hypertonic sodium chloride 20% at 5 minutes, had protoscolicidal effect; while silver nitrate 0.5% did not have a complete protoscolicidal effect up to 5 minutes.

## ASSESSMENT OF TRICLABENDAZOLE TREATMENT AGAINST *E. MULTILOCULARIS*

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**Relevance.** *Echinococcus multilocularis* causes the severe zoonotic disease alveolar echinococcosis (AE) for which treatment is currently limited [1]. The regenerative potential of *E. multilocularis* contributes to conventional albendazole/mebendazole-based therapy only being parasitostatic and thus novel treatment options are needed [2].

**Materials and methods.** We tested several benzimidazoles against *in vitro* cultured *E. multilocularis* metacestodes, which represent the disease-causing stage of AE, and against isolated germinal layer cells, which are responsible for the regenerative potential of the parasite [3]. Triclabendazole (TCBZ) hereby exhibited promising *in vitro* activity, and therefore, we tested it in the secondary AE mouse model. Six weeks after intraperitoneal infection, animals were treated five days per week by multipipette-guided drug administration [4], a newly introduced method that trains for voluntary ingestion of tested drugs. We assessed the standard drug albendazole (ABZ, 200 mg/kg), TCBZ (100 mg/kg), a combination of both these drugs (2 weeks / 1 week), and a placebo control. After treatment for 12 weeks, parasite weight and viability of the resected parasite material was assessed. In addition, blood samples were taken, 1h, 4h, and 7h after treatment to measure drug levels and metabolism of the drugs.

**Results.** MDA was successfully applied in this study with most animals showing high compliance. Neither parasite weight analyses, nor *in vitro* culture of the resected parasite material showed an improved treatment outcome of TCBZ-based treatment when compared to ABZ alone. To test parasite *in vivo* viability, mice were reinfected with the parasite material. There was no reduction observed in infectivity. Histological analyses of mouse spleens, kidneys and livers revealed no pathological alterations induced by drug-treatment. Assessment of plasma metabolite levels showed TCBZ to be metabolized significantly slower and taken up more efficiently than ABZ. This resulted in an increased concentration of the active metabolite TCBZ-sulfoxide in the blood of AE mice.

**Conclusion.** Under the conditions tested, TCBZ treatment was not active against AE *in vivo*. TCBZ does, however, show a differing pharmacokinetic profile to ABZ, with increased plasma levels of metabolites. Follow-up studies showed that TCBZ metabolite concentrations in vesicle fluid of successfully *in vitro* treated metacestodes is 100-fold lower than what was measured in plasma of treated AE mice. This might hint at an insufficient uptake of the drug into metacestodes hindering a successful outcome of the *in vivo* treatment.

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## MANAGEMENT OF BILIARY OBSTRUCTION IN PATIENTS WITH NEWLY DIAGNOSED ALVEOLAR ECHINOCOCCOSIS: A SWISS RETROSPECTIVE COHORT STUDY

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**Aim.** To compare effectiveness and safety of conservative and interventional treatment approaches in patients with newly diagnosed AE and biliary obstruction.

**Methods.** AE patients treated at two referral centers in Switzerland, presenting with hyperbilirubinemia at diagnosis were included, unless another underlying etiology was identified. Groups were divided depending on whether patients initially received biliary tract intervention or not. Primary study endpoint was the normalization of bilirubin levels within a six-month period. Secondary endpoints included i.a. the occurrence biliary complications and duration of hospital stay.

**Results.** 28 patients were included, 17 patients received benzimidazole therapy alone and 11 patients additionally biliary tract intervention. Baseline characteristics did not differ between groups. All patients but one in each group achieved the primary study endpoint ( $p=0.747$ ). Biliary tract intervention was associated with faster laboratory improvement, but also with more frequent early biliary complications and longer hospital stay.

**Conclusion.** Biliary obstruction in patients with newly diagnosed AE can be treated effectively with benzimidazole therapy alone. Biliary tract intervention is associated with a high complication rate and should probably be reserved for patients with insufficient response to benzimidazole therapy.

## GIANT UNOPERABLE PARASITIC CYST OF THE SPLEEN TREATED WITH PAIR AND CONTINUOUS CATHETERIZATION

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**Introduction.** Splenic CE is rare. Three treatment options exist for CE: surgery, medical therapy and percutaneous treatments [1,2]. We describe a 50 y.o. patient with a giant splenic parasitic cyst successfully treated with continuous catheterization as surgery was contraindicated and medical treatment ineffective.

**Case report.** The patient had severe obesity (BMI 40.8), hypertension, atrial fibrillation, chronic renal disease, prosthetic aortic valve and was on oral anticoagulant. He reported previous surgery for liver, omental, pelvic CE and abdominal hernia. Eleven years after the first surgical intervention, a CT scan revealed a CE3a giant (18.5x13.5 cm) cyst in the spleen [3]. Splenectomy was excluded (high surgical risk related to the multiple comorbidities), while partial splenectomy and laparoscopic approach were not considered because of the potential cyst rupture. We used the percutaneous approach: an 8F pigtail catheter was inserted under US guidance in the cyst, the fluid aspirated, 20 % hypertonic saline was injected in the cavity and re-aspirated. The catheter was left *in situ* for

spontaneous drainage for five days in association with ABZ [4]. Although the cyst size was not permanently reduced, we obtained the sterilization of the cyst, with no viable protoscolices found after lavage with saline.

**Results.** The procedure was repeated four times over a 10 year-period, without any complications. ABZ administration was discontinued in 2009 and the annual US follow-up showed a smaller splenic cavity (7.2x6.8 cm). The patient died of unrelated causes (myocardial infarction) in 2013.

**Conclusions.** Although unconventional and failing to obtain solidification (CE4), this treatment was instrumental in keeping the patient safe: cavity sterilization virtually makes secondary echinococcosis impossible, even in case of rupture. Until today only ten studies report PT of splenic cysts, none of which with continuous drainage. Further experience is needed to confirm its safety and efficacy of this treatment under less extreme circumstances.

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## ORAL DELIVERY OF AGENT-LOADED PLGA NANOPARTICLES: INCREASING THE DRUG CONCENTRATION IN THE LIVER FOR IMPROVING THE THERAPEUTIC EFFICACY AGAINST HEPATIC ALVEOLAR ECHINOCOCCOSIS

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**Relevance.** Alveolar echinococcosis (AE) is a lethal parasitic disease which primarily affects the liver. Although much effort has been made to develop new drugs against this neglected disease, the current treatment options remain limited and new drug delivery is considered as a new path-breaking for the treatment.

**Materials and methods.** Nanoparticles (NPs) have gained much attention in the field of drug delivery due to their potential improvement of delivery efficiency and targetability. In this study, biocompatible PLGA nanoparticles encapsulating a novel carbazole aminoalcohol anti-AE agent (H1402) identified in our previous study [1] were prepared to promote the delivery of the parent drug to liver tissue for treating hepatic AE.

**Results.** H1402-loaded nanoparticles (H1402-NPs) had a uniform spherical shape with a mean size of 55 nm. Compound H1402 was efficiently encapsulated into PLGA NPs with a maximal encapsulation efficiency of 82.1% and drug loading content of 8.2%. An *in vitro* uptake assay demonstrated that H1402-NPs rapidly penetrated the vesicle wall and extensively accumulated in the metacystode vesicles of *Echinococcus multilocularis* within only 1 h. The biodistribution profile of H1402-NPs determined through *ex vivo* fluorescence imaging revealed significantly enhanced liver distribution compared to unencapsulated H1402, which translated to the improved therapeutic

efficacy and reduction of systemic toxicity (especially hepatotoxicity and cytotoxicity) in a hepatic AE murine model. Following a 30-day oral regimen (100 mg/kg/day), H1402-NPs significantly reduced the parasitic burden in both the parasite mass (liver and vesicle total weight, 8.8%) and average vesicle size (89.9%) compared to unmedicated infected mice (both *p-values* < 0.05); the treatment outcome was more effective than those of albendazole- and free H1402-treated individuals.

**Conclusion.** Encapsulated H1402 into PLGA nanoparticles increase the concentration of H1402 in the liver and improve the therapeutic efficacy of this drug against hepatic AE.

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## СЛОЖНОСТИ И ТРУДНОСТИ В ЛЕЧЕНИИ И ПРОФИЛАКТИКЕ МНОЖЕСТВЕННОГО И СОЧЕТАННОГО ЭХИНОКОККОЗА

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**Введение.** Большинство рецидивных форм заболевания эхинококкозом некоторые ученые связывают чаще всего с множественным и сочетанным поражением органов, которые являются непосредственными источниками этой причины [1,2]. И, несмотря на последние достижения современной медицины, число этих форм заболеваемости, наряду с его повторным развитием, к сожалению, остается на высоком уровне и решение проблем их профилактики и лечения является одной из сложных и по сей день малоизученных [3,4,5].

**Цель работы.** Определить непосредственную связь в развитии рецидивов множественных и сочетанных форм эхинококкоза.

**Материал и методы.** Обследованы 62 пациента с множественным и сочетанным эхинококкозом, у 8 из которых установлен диагноз множественный рецидивный эхинококкоз. Изолированное поражение печени, где отмечались две и более кисты различного диаметра, наблюдались у 18 больных, причем у основного их количества характер кист соответствовал стадии живого паразита. 15 пациентов госпитализированы с сочетанным поражением печени и легких, при которых количество паразитарных кист составляло 3-4 и более, максимальное число некоторых достигало не менее 12 кист. Остальные 5 больных находились с сочетанно-множественным эхинококкозом печени, легких и других органов брюшной полости. Всем больным были проведены оперативные методы лечения и в разные сроки после проведенной операции рекомендовали профилактическую химиотерапию. На всех этапах по показаниям проводились инструментально-лабораторные методы исследования, которые и указывали на наличие (или отсутствие) гидатидозных кист, их локализацию, количество, размеры и стадию жизнедеятельности паразита, что было важным в выборе дальнейшей тактики лечения.

**Результаты и их обсуждение.** Согласно наблюдениям, у подавляющего большинства пациентов отмечались рецидивы заболевания, трактуемые как истинный и резидуальный эхинококкоз. Общее число рецидивного эхинококкоза, который отмечался в сроки от 1,5-2х до 4х лет, составило 18 человек, что соответствует 29%, резидуального – у 7 (11,3%). человек. У некоторых пациентов на фоне большого количества кистозных образований определить их точное число не удавалось, ввиду их малых размеров и других причин, что в итоге явилось одной из причин развития рецидивов эхинококкоза. Всем больным в послеоперационном периоде назначался курс антипаразитарной терапии по общепринятой схеме, несмотря на которую, 9 пациентов оперированы повторно в различные сроки.



**Выводы.** В решении вопросов возникновения рецидивов эхинококкоза при множественных и сочетанных его формах важным являются до- и послеоперационные лечебно-профилактические мероприятия, которые в комплексном их проведении имеют влияние на качество жизни пациентов в ближайшем и отдаленном периодах.

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## ADVANCING IN DRUG DISCOVERY AGAINST ECHINOCOCCUS GRANULOSUS SENSU STRICTO: DEVELOPMENT AND UTILIZATION OF IMPARTIAL IN VITRO SCREENING ASSAYS

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*Echinococcus multilocularis* and *E. granulosus s.l.* are the causative agents of alveolar and cystic echinococcosis, respectively. Drug treatment options for these diseases are limited to benzimidazoles, which are not always efficacious, and adverse side effects are reported. Thus, novel and improved treatments are needed. In this study, the previously established platform for *E. multilocularis in vitro* drug assessment was adapted to *E. granulosus s.s.* *In vitro* culture protocols for *E. granulosus s.s.* were established, and drug susceptibilities of both species were compared employing established assays. MMV665807, niclosamide and nitazoxanide were active in all assays against both species. Albendazole displayed activity against *E. multilocularis* GL cells, but no effects were seen in albendazole-treated *E. granulosus s.s.* Treatment with albendazole and monepantel had no impact on protoscoleces motility. In conclusion, *in vitro* culture techniques and drug screening methods previously established for *E. multilocularis* were successfully implemented for *E. granulosus s.s.*, allowing comparisons of drug efficacy between the two species. This study provides *in vitro* culture techniques for the reliable generation of *E. granulosus s.s.* metacestode vesicles and GL cell cultures and describes the validation of standardized *in vitro* drug screening methods for *E. granulosus s.s.*

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**ALBENDAZOLE TREATMENT OF ALVEOLAR  
ECHINOCOCCOSIS: A COHORT STUDY****Gulnara Mynbaeva**

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**Materials and Methods.** Study patients were identified in a cross sectional study in the Alay Rayon in Osh Oblast in South Kyrgyzstan. A second group of subjects was identified through an additional cross sectional study in Kochkor Rayon, Naryn Oblast. Based on the ultrasound results patients were selected as assigned to the trial group, advised on further treatment or advised that there was no evidence of AE lesions in their livers.

**Results.** This study documents a cohort study to investigate the efficacy of albendazole treatment for early cases of alveolar echinococcosis, the efficacy in more advanced cases and of wait and see management. The wait and see protocol indicates that over a relatively short period of time, the majority of lesions showed little sign of progression. Thus 77% of patients with small P1N0M0 lesions showed no signs of progression during the observation period. However, there was evidence in the remaining 23% of patients in this group. This suggests that wait and see is a viable option, but there is a significant chance that patients will require albendazole therapy.

**EVALUATION OF STRUCTURAL CHANGES OF ECHINOCOCCUS GRANULOSUS  
SENSU STRICTO PROTOSCOLECES FOLLOWING EXPOSURE TO DIFFERENT  
PROTOSCOLICIDAL SOLUTIONS EVALUATED BY DIFFERENTIAL INTERFERENCE  
CONTRAST (DIC)/NOMARSKI MICROSCOPY**

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The lethal efficacy of protoscolicidal agents is usually evaluated by vital stains, which is occasionally questionable. The present study assessed structural changes of protoscoleces of *E. granulosus* s.s. following exposure to different natural and chemical protoscolicidal agents by (DIC)/Nomarski Microscopy. Individually, about 1000 protoscoleces were exposed to 0.5% silver nitrate, 20% hypertonic saline solution, 0.5% cetrimide solution and two different concentrations of garlic chloroformic extraction as well as PBS in triplicate. The results revealed the degeneration of the tegument, disorganization of the hooks, and reduction of the size of the protoscoleces exposed to cetrimide, hypertonic sodium chloride, and silver nitrate. Calcareous corpuscles became blurred and opaque and their numbers decreased except PBS. Cetrimide 0.5% and hypertonic sodium chloride 20% caused more pronounced structural changes in the exposed protoscoleces. These changes demonstrated by DIC microscopy can be used as a supplementary tool for evaluating the protoscolicidal agents.

## HUMAN ALVEOLAR ECHINOCOCCOSIS: A LONG-TERM FOLLOW UP OF PATIENTS AT THE TERTIARY CARE CENTRE IN PRAGUE, CZECH REPUBLIC

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**Relevance.** Human alveolar echinococcosis (AE) is being recognized as an emerging zoonotic infection in the Czech Republic during last two decades. The objective of our presentation is to describe clinical and laboratory characteristics, management and outcome of patients with AE treated in our hospital.

**Method.** We are presenting a prospective descriptive study of 25 AE cases treated at the Department of Infectious Diseases in University Hospital Bulovka in Prague since 2012. Epidemiological, clinical and laboratory parameters has been collected and evaluated in all patients.

**Results.** There have been managed total 25 patients (16 females and 9 males) with AE at our department since 2012. Majority of cases were diagnosed in Czechs (21), 3 females were from Ukraine and one female from Slovakia. The median age of our patients was 48 years at the time of diagnosis. Female age ranged between 7 and 73 years, male age was between 22 and 81 years. Lesions were localized in liver only in 17 patients (B67.5 according WHO ICD-10 classification), in liver with propagation to adjacent organs in 5 patient, in liver with multiple metastasis to lung or peritoneum and abdominal wall in 3 patients (B67.6). One patient acquired infection with infective graft after liver transplantation. 5 of 24 AE patients were immunosuppressed at the time of diagnosis (transplantation of liver or kidney, sclerosis multiplex, myasthenia gravis, Bechterew's disease). Serology for echinococcosis was positive (20) or borderline (4) in all patients, except one infected with liver AE lesion after transplantation. There was not significant eosinophilia, but total IgE was elevated in 13 of 23 patients. The curative treatment is based on surgery and long-term continuous albendazole (ABZ) administration. Inoperable lesions are presented in 10 patients, nine of them are on ABZ treatment, one man is not tolerating ABZ and treatment with praziquantel is suggested. In total, 11 patients underwent successful liver resection, treatment with ABZ was discontinued in three of them 3 to 7 years after surgery. In one patient, resection of large liver AE mass and heterotopic auxiliary liver transplantation was performed in August 2021. After liver regeneration, graftectomy was performed in March 2022 and immunosuppressive treatment was discontinued. In addition, 2 female patients underwent liver transplantation. ABZ treatment was discontinued after 3 years in one of them, before she become pregnant and delivered healthy girl. Clinical stage of all patients is favorable, except for two who died from AE unrelated reason.

**Conclusions.** AE has an increasing incidence in the Czech Republic with 3 to 5 new cases diagnosed each year. Nearly one half of them are treated at our department. Management of AE is multidisciplinary at specialized infectious disease units with long-term follow up after successful surgery.

## PRELIMINARY STUDY ON THE CURRENT SITUATION OF PREVENTION AND TREATMENT FOR HYDATID PATIENTS AND THE MODEL OF COMBINED THERAPY AND PREVENTION IN XINJIANG EPIDEMIC AREAS

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**Abstract.** Xinjiang has the largest number of echinococcosis endemic counties, 98 counties and cities in 14 prefectures of Xinjiang have reported echinococcosis patients, and about 50 counties and cities are threatened. In this work, We investigated the current status of drug therapy in patients with echinococcosis endemic areas in Xinjiang, summarized the implementation effect and existing problems of the combined mode of medicine and prevention, and put forward evaluation criteria.

**Methods.** Data of hydatid patients registered in designated hospitals and CDC of Qitai, Jimusar and Chabuchaer were collected. Follow-up was conducted on the whole treatment process of hydatid patients in the first pilot counties, and comparative analysis was made on the treatment situation before and after the the model of combined hydatid therapy and prevention. Bagging model, AdaBoost model, Random Forest model and Logistic regression model were used to analyze the indicators that might affect the follow-up effect. All the respondents signed the informed consent.

**Results.** Hydatid patients were mainly distributed in rural areas (85.9%), most of them were in high school or below (96.3%) and 55.6% (75/135) were relapsed and treated again. 34.8% (47/135) of the follow-up medication did not meet the standard. Analysis of influencing factors of effective follow-up medication in patients with hydatid showed that age, occurrence of adverse drug reactions and times of administration had great influence on the effective follow-up medication rate. After the implementation of the model of combined hydatid therapy and prevention, the proportion of unknown diagnosis stages of Cystic Echinococcosis in Jimusar decreased by 26.9%.

**Conclusion.** Hydatid patients had high recurrence rate in Xinjiang, and the effective follow-up medication rate is low. The pilot work has initially formed a linkage mechanism between medical institutions and CDC institutions, and the accuracy of patient diagnosis has been improved, but the working model still needs to be improved.

## DEVELOPING WHO GUIDELINES FOR TREATMENT OF PATIENTS WITH CYSTIC ECHINOCOCCOSIS

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**Relevance.** Human cystic echinococcosis (CE) is caused by the development of *Echinococcus granulosus sensu lato* metacestodes, mainly in liver and lungs. CE is often expensive and complex to manage clinically, sometimes requiring extensive surgery and/or prolonged drug therapy. Several clinical management options are described for CE, and the choice is primarily based on the organ affected and the imaging characteristics of the cyst, following a stage-specific approach, in addition to available healthcare infrastructure and medical expertise. In 2010, the World Health Organizations' Informal Working Groups on Echinococcosis (WHO-IWGE) issued an Expert Consensus for diagnosis and treatment of cystic and alveolar echinococcosis [1], which requires updating. For this, and to foster a more widespread uptake and application of best practices in the management of human CE, WHO has now initiated the process to develop official guidelines, to provide guidance on the choice of clinical management, ensuring patients can be offered and receive the most appropriate care.

**Materials and methods.** Official WHO guidelines are developed in a systematic process to ensure using the best available evidence to minimize bias while maximizing transparency and usability [2]. In early 2022, a guideline development group (GDG) constituting of 18 international experts covering all WHO Regions has been established through an open "Call for Experts". To develop the recommendations, specific PICO (population, intervention, comparison, outcomes) questions for areas with equipoise in active treatment of uncomplicated liver and lung CE cysts were developed. Decisions will be made following systematic rating of evidence. Great care will be taken to ensure that the recommendations mirror the capabilities of the various settings. The ongoing work of the GDG will be presented.

**Results.** The guideline will enable clinicians in their respective healthcare environments to manage CE patients appropriately and at the highest standards of care possible by providing recommendations on the indications of the four main management modalities: (1) anti-parasitic drug treatment, (2) percutaneous methods, (3) surgery, (4) "watch & wait" depending on the stage and location of the cysts.

**Conclusion.** It is expected that clear and appropriate guidance will reduce and avoid over- and mistreatment of patients ensuring the highest standard of healthcare and well-being for patients.

We acknowledge all members of the Guideline Development Group. The list of experts including their biographies can be found [here](#)

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## CASE REPORT. INFREQUENT COMPLICATION OF HEPATIC CYSTIC ECHINOCOCCOSIS (CE): HEPATOTHORACICTRANSDIAPHRAGMATIC EXTENSION

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**Introduction.** Hepatothoracictransdiaphragmatic extension (HTTE) of hepatic cystic echinococcosis (HCE) is a rare complication of hepatic CE occurring up to 0.16 to 16% [1]. The rupture can occur into pleural cavity, the bronchus or lung parenchyma. This complication has a mortality around 14% [2].



**Case presentation.** A 93-year-old man with a previous history of complicated hepatic CE opened to biliary tree treated with percutaneous drainage and another 2 giant hepatic CE that previously have refused to surgery, was admitted in emergency service due to a cough with mucopurulent sputum, right chest pain in the dorsal region, breathlessness, and tachycardia, without leukocytosis or fever, with amagnetic resonance imaging (MRI) with diagnosis of HTTE of HCE grade 3 [3].

An emergency 26 French drain was placed in both, right thoracic cavity and hepatic cyst through a 10 mm laparoscopic safety shielded tip trocar drained daughter vesicles, rest of membranes, and cloudy fluid, which alleviated the symptoms. In the postoperative days, drainage of material frequently obstructed the pleural drainage that also had a cloudy color. With a diagnosis of empyema and undrained and unresolved hepatothoracic communication, video-assisted thoracic surgery (VATS) was performed with pleuropulmonary empyemectomy, irrigation and drainage of the hepatic cyst and pleural cavity, diaphragmatic suturing, drainage of the cystic and pleural cavity. The patient was discharged in the 11th postoperative day and is under control and taking albendazole.

**Discussion.** Hepatothoracic transdiaphragmatic extension of hepatic cystic echinococcosis is a rare and life-threatening complication of hepatic CE. In the imaging diagnosis of CE, MRI with heavily T2-weighted series is preferable to computed tomography in case of ultrasound cannot be performed due to cyst location as this case [4]. Surgery is the treatment of choice in complicated cystic echinococcosis. In this case, all the approach initial and definitive treatment was done by minimally invasive surgery.

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## WATCH AND WAIT FOR UNCOMPLICATED CE4 AND CE5 ECHINOCOCCAL CYSTS OF THE LIVER. TWELVE-YEAR EXPERIENCE FROM 2 CENTERS IN ITALY

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**Relevance.** Cystic Echinococcosis (CE) is a chronic, neglected, and complex infection caused by the tapeworm *E. granulosus* *sl* causing the formation of parasitic cysts[1]. The liver is most frequently involved [2]. The clinical management of these patients should follow a stage-specific approach as recommended by the WHO Informal Working Group on Echinococcosis[3]. Uncomplicated, inactive (CE4, CE5) liver cysts can be managed with ultrasound monitoring alone over time[3]. This watch and wait strategy spares the patients unnecessary treatments and allows better resource allocation for healthcare systems[4]. However, data on the long-term safety of this strategy are still scant[5].

**Materials and Methods.** We collected data on liver CE patients from two Italian centers and recorded in the European Registry of Cystic Echinococcosis (ERCE) between 2010 and 2022. Inclusion criteria were the presence of at least one inactive uncomplicated hepatic cyst with a minimum follow-up of 24 months and with at least a yearly ultrasound visit.

**Results.** One-hundred patients with a total of 140 cysts were eligible: 65 were spontaneously inactive (SI), 75 were inactive after medical or percutaneous treatment (IAT). Only one cyst (1.3%) in the IAT group showed a reactivation (growth of daughter cysts) after 1 year of monitoring. All cysts in the SI group stayed inactive. The mean follow-up time was 53.3 months (SD 25.5) in the spontaneously inactive group and 60.0 months (SD 32.8) in the inactive after treatment group.

**Conclusion.** Our study supports the safety of “watch and wait” approach of uncomplicated inactive liver cysts. Unlike other studies[6], no difference in the reactivation rate between treated and untreated cysts was found.

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## CHARACTERISTICS OF POSTOPERATIVE RECURRENCE OF HEPATIC ALVEOLAR ECHINOCOCCOSIS AND RECURRENCE FACTORS DISCUSSED

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**Objective.** Hepatic alveolar echinococcosis (HAE) grows invasively, and most of them have already progressed to advanced stages at the time of consultation, which makes surgery difficult and has a high rate of postoperative recurrence. The aim of this study was to investigate the characteristics of postoperative recurrence of HAE and the factors that influence recurrence through recurrent cases.

**Methods.** A total of 54 patients who underwent surgical treatment after diagnosis of HAE and were followed up after surgery from January 2017 to June 2023 were included in this study, of which 21 radical resections (38.9%) and 33 palliative resections (61.1%) were performed; 27 had postoperative recurrences, and 27 did not have any recurrences after surgery; 26 males (48.2%), 28 females (51.8%) , age 11-68 years old, mean age  $39.07 \pm 14.50$  years old; all patients underwent preoperative CT scanning, 36 MR scans, CT scanning at the time of recurrence, and 12 MR scans; inclusion factors: gender, age, site of primary lesion, size, number, whether bile ducts were invaded, whether intrahepatic macrovessels were invaded, P staging, type of lesion, whether it was metastasized prior to surgery, and surgical modality Recorded the time of lesion recurrence, recurrence site; logistic regression was used for univariate and multivariate statistical analyses.

### Results:

1. The recurrence time was 1-116 months after surgery, with a mean recurrence time of  $33.04 \pm 28.64$  months;

2. 33.3% of the lesions were recurrent in the surgical stump, and 16.7% of the lesions recurred in other hepatic segments;

3. Among the 27 recurrent cases, 12 cases (44.4%) had primary lesions invading both halves of the liver, 16 cases (59.3%) had the highest proportion of P4 stage, 25 cases (92.6%) had vascular invasion, and 19 cases (70.4%) underwent palliative resection; univariate analysis showed that lesion invasion of both halves of the liver, vascular invasion, late P staging, and palliative surgery were associated with postoperative recurrence of HAE ( $P < 0.1$ ), and multifactorial analysis showed that vascular invasion could independently affect the recurrence of HAE lesions ( $P < 0.05$ ).

**Conclusion.** Lesion invasion of both halves of the liver, vascular invasion, late P staging, and palliative surgery increase the risk of postoperative recurrence, with vascular invasion being is a major risk factor influencing the recurrence of the lesion; and there is a high incidence of surgical stump recurrence.

## IDENTIFICATION AND METABOLIC PATHWAY ANALYSIS OF HARMINE DERIVATIVE H-2-104 IN RATS BASED ON UPLC-Q-TOF-MS TECHNOLOGY

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**Relevance.** *Peganum harmala* L. is a characteristic plant medicine in Xinjiang, and its main alkaloid harmine has good anti-hydatid activity, but obvious neurotoxicity limits its application. Therefore, research group synthesized a variety of derivatives with harmine as the lead compound. H-2-104, a candidate drug with high efficiency and low toxicity was obtained through pharmacodynamic screening and safety evaluation in vitro and in vivo. In this study, UPLC-Q-TOF-MS was used to identify the metabolites of H-2-104 in rats, and to explore its possible metabolic pathways, so as to lay the experimental foundation for the development of new drugs anti-hydatid disease.

**Materials and methods.** Twelve healthy SD rats, half male and half female, were given H-2-104 by gavage. Plasma, bile, urine and feces samples were collected at different time. Gradient elution

was performed with acetonitrile (A) -0.1% formic acid aqueous solution (B) as the mobile phase. The analysis was carried out in the positive ion mode of the ESI. According to the relative retention time, mass-to-charge ratio, excimer ion peak and characteristic fragment ion of the obtained compounds, the metabolites were identified and their possible metabolic pathways were explored.

**Results.** A total of 46 metabolites of H-2-104 were identified, including 24 in blood, 22 in bile, 29 in feces and 19 in urine. There were 14 metabolic pathways involved, including monohydroxylation, dihydroxylation, demethylation, glucuronidation, and sulfation. The results also showed that fecal excretion was the main route of H-2-104 and its metabolites, followed by bile.

**Conclusion.** The metabolites and metabolic pathways of H-2-104 in rats were systematically elucidated for the first time, which laid an experimental foundation for the subsequent research and development of harmine derivatives.

**Key words:** harmine derivatives, metabolites, metabolic pathways.

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## IMPROVEMENT OF ANTIALVEOLAR ECHINOCOCCOSIS EFFICACY OF NOVEL ALBENDAZOLE-BILE ACIDS DERIVATIVES WITH ENHANCED ORAL BIOAVAILABILITY

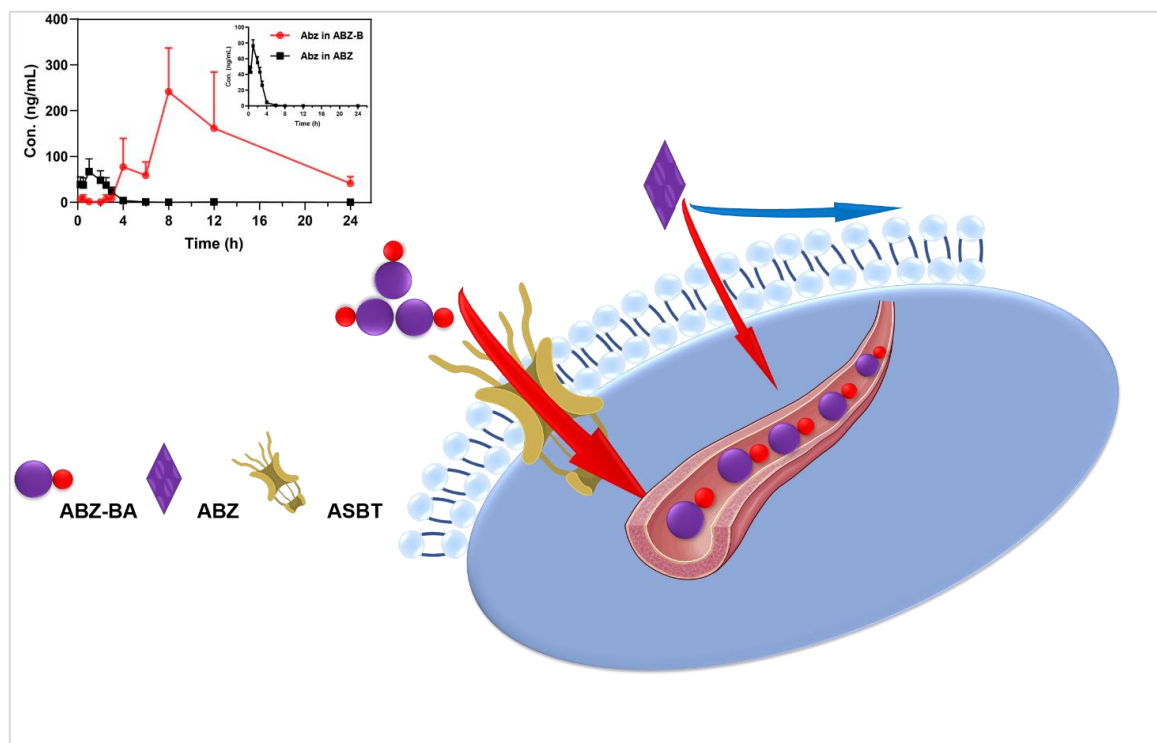
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Alveolar echinococcosis (AE) is a chronic and fatal infectious parasitic disease, which has not been well-researched. Current recommended therapies for AE by the World Health Organization include complete removal of the infected tissue followed by two years of albendazole (ABZ), administered orally, which is the only effective first-line anti-AE drug. Unfortunately, in most cases, complete resection of AE lesions is impossible, requiring ABZ administration for even longer periods. Only one-third of patients experienced complete remission or cure with such treatments, primarily due to ABZ's low solubility and low bioavailability. To improve ABZ bioavailability, albendazole bile acid derivative (ABZ-BA) has been designed and synthesized. Its structure was identified by mass spectrometry and nuclear magnetic resonance. Its physicochemical properties were evaluated by wide-angle X-ray diffraction, differential scanning calorimetry, scanning electron microscopy, and polarizing microscopy; it was compared with ABZ to assess its solubilization mechanism at the molecular level. To avoid the effects of bile acid on the efficacy of albendazole, the inhibitory effect of ABZ-BA on protoscolex (PSCs) was observed *in vitro*. The inhibitory effect of ABZ-BA on PSCs was evaluated by survival rate, ultrastructural changes, and the expression of key cytokines during PSC apoptosis. The results showed that ABZ-BA with 4-amino-1-butanol as a linker was successfully prepared. Physicochemical characterization demonstrated that the molecular arrangement of ABZ-BA presents a short-range disordered amorphous state, which changes the drug morphology compared with crystalline ABZ. The equilibrium solubility of ABZ-BA was 4-fold higher than ABZ *in vitro*. ABZ-BA bioavailability in Sprague-Dawley (SD) rats was 90-fold higher than ABZ *in vivo*. The inhibitory effect of ABZ-BA on PSCs was identical to that of ABZ, indicating that adding bile

acid did not affect the efficacy of anti-echinococcosis. In the pharmacodynamics study, it was found that the ABZ-BA group had a significant therapeutic effect after 1 month of oral administration. The oral bioavailability of ABZ-BA is significantly better than ABZ due to the transformation of the physical state from a crystalline state to an amorphous state. Furthermore, sodium-dependent bile acid transporter (ASBT) expressed in the apical small intestine has a synergistic effect through the effective transport of bile acids. Therefore, we concluded that the NC formulation could potentially be developed to improve anti-AE drug therapy.



Scheme 1. Improving the efficacy of albendazole (ABZ) against alveolar echinococcosis (AE) by an optimized formulation. An optimized formulation containing Albendazole-bile derivative(ABZ-BA) was developed, which significantly improved the pharmacokinetics and the anti-AE efficacy, after a 30-day, once-daily oral administration.



## РЕЗУЛЬТАТЫ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ ЭХИНОКОККОЗА ЛЕГКИХ

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**Актуальность.** Эхинококкоз – это достаточно распространенное во всем мире эпидемиологическое паразитарное заболевание, приносящее огромный ущерб здоровью людей [1]. Эхинококкоз является весьма актуальной проблемой в связи с большим числом больных и существованием эндемических регионов, куда относится и территория Кыргызской Республики [2]. По частоте поражения эхинококкозом легкие занимают второе место после печени и составляют 10-40% всех локализаций. До настоящего времени оперативное лечение эхинококкоза легких считается единственным радикальным методом [3].

**Цель исследования.** Проанализировать результаты хирургического лечения эхинококкоза легких по данным Национального хирургического центра им. М.М. Мамакеева.

**Материалы и методы.** Представлены результаты хирургического лечения 396 больных эхинококкозом легких в Национальном хирургическом центре им. М.М. Мамакеева за период с 2003 по 2022 гг. Мужчин было 242 (61,2%), женщин – 154 (38,8%) в возрасте от 15 до 72 лет.

При установлении диагноза эхинококкоза легких использовался диагностический протокол (сбор жалоб, анамнез заболевания, эпидемиологический анамнез, лабораторные, неинвазивные инструментальные и инвазивные видеоэндохирургические методы), рентгенологическое и компьютерно-томографическое исследование грудной клетки. С целью подтверждения кистозного образования и в легком и исключения эхинококкоза других органов проводилось ультразвуковое исследование плевральной и брюшной полостей.

**Результаты и обсуждение.** За последние 20 лет отмечается динамика к увеличению заболеваемости эхинококкозом легких. За период с 2003 по 2012 год было выявлено 180 (45,5%) больных, за период с 2013 по 2022 год было выявлено 216 (54,5%) больных эхинококкозом легких. Эхинококкоз правого легкого диагностирован у 234 (59,1%) больных, эхинококкоз левого легкого – у 129 (40,9%) и двусторонний эхинококкоз – у 23 (7,1%) больных. 281 (71%) больных были госпитализированы и прооперированы в плановом порядке после полного обследования. 115 (29%) больных госпитализированы в экстренном порядке с развившимися уже осложнениями. В структуре которых нагноение эхинококковой кисты без нарушения целостности фиброзной оболочки выявлено у 37 (32%) больных, с прорывом кисты в бронх – у 49 (43%), в плевральную полость – у 23 (20%), одновременно в бронхи и в плевральную полость – у 6 (5%) больных. Традиционная переднебоковая или боковая торакотомия по пятому межреберью выполнена всем больным. В послеоперационном периоде

осложнения развились у 83 (21%) больных, которые ликвидированы консервативным и пункционным методами. Повторные хирургические вмешательства в виде реторакотомии выполнены у 4 (1,01%) больных, редренирования – у 3 (0,75%) больных. Летального исхода не было.

**Заключение.** За последние 20 лет отмечается тенденция к увеличению заболеваемости эхинококкозом легких, осложнения которого приводят к тяжелым последствиям опасным для жизни больного и требуют неотложной медицинской помощи. Комплексное обследование больных с применением современных методов визуализации и видеоэндохирургических методов повышает эффективность диагностики и хирургического лечения эхинококкоза легких.

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## **РЕТРОСПЕКТИВНЫЙ АНАЛИЗ РЕЗУЛЬТАТОВ ОПЕРАТИВНОГО ЛЕЧЕНИЯ БОЛЬНЫХ АЛЬВЕОКОККОЗОМ ПЕЧЕНИ**

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**Введение:** альвеококкоз – заболевание вызванное *echinococcus multilocularis*, характеризующиеся инфильтративным ростом. Основным методом лечения является хирургический, с последующей химиотерапией. Большинство больные обращаются с распространёнными формами, когда возникли осложнения. Многие исследователи указывают, что радикальные операции не превышают 35-40% и после них возникают осложнения в 60-65%.

**Цель исследования:** провести анализ результатов оперативного лечения больных альвеококкозом печени на базе одной клиники.

**Материалы и методы.** Под наблюдением было 751 больных которые находились на стационарном лечении в хирургических отделениях ГКБ №1, в период с 2000 по 2022 годы. Из 751 больных женщин было 459 (61,1%), мужчин 292 (38,9%). Средний возраст составил 25,0±1,5 лет. Поражение правой доли печени 425 (56,5%), левой доли 251 (33,4%), обе доли 75 (10,1%). У 13 больных отмечены метастазы в легкие, у 8 в головной мозг, у одной метастазы в правый желудочек сердца и легкие. Также у одного больного отмечалось редкое сочетание альвеококкоза с эхинококкозом. По классификации ВОЗ 45-48% с P3-P4 и 52-55% с P1-P2. Умерло 16 (2,15%) больных после оперативных вмешательств.

**Результаты.** Из 751 больных альвеококкозом печени, радикальные операции выполнены у 570 (54,4%), паллиативные у 181 (24,5%), а 22 больных нуждаются в аутоотрансплантации и трансплантации печени. Все возникшие осложнения после радикальных операции распределили по классификации Clavien-Dindo. Наибольшую группу составили больные II степени и III-а степени, которым в лечении осложнений после операции требовались трансфузии, реже отмечана IV-а и IV-b –недостаточность одного органа или полиорганная и V

степень составила 14,2% из числа всех возникших осложнений. А больным, после паллиативных операций мы осложнения рассмотрели согласно классификации Clavien-Dindo, где большой процент был IIIa степени и IVb степени, V составила 15,3%.

**Заключение:** результаты оперативного лечения показали, что число поступивших больных альвеококкозом с каждым годом растет и на базе одной клиники показано большое число наблюдений. Выполнение радикальных операций при альвеококкозе печени хотя и представляют сложности, но при своевременной диагностике, когда нет осложнений можно добиться положительных результатов. Паллиативные операции в комбинации с химиотерапией альбендазолом оправдано.

**Ключевые слова:** альвеококкоз, операции, летальность.

## DELAYED SURGICAL TREATMENT OF A CE1 LUNG CYST RESULTING IN PERICYSTECTOMY OF CE4 CYST

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**Introduction.** Lung is the second most common localization of Cystic Echinococcosis (CE) [1,2]. Surgery is the primary treatment, with a minor role for medical therapy. Delayed diagnosis and treatment may have important consequences [3,4]. We present a case of lung CE in which the delayed surgical treatment due to COVID epidemic led to a CE4 cyst requiring pericystectomy.

**Case presentation.** The patient, a 36 y.o. Ghanaian man, was admitted to the E.D. in December 2019, following a car accident. A CT scan showed a 11x7 cm cystic lesion located in the superior lobe of the right lung. Serology for *E. granulosus* tested positive and diagnosis of lung CE was made. Medical therapy was started with albendazole (ABZ), since the COVID-19 epidemic made surgery unavailable. After two episodes of vomica (February 2020 and April 2021), lung CT scan showed major changes in cyst appearance (wall thickening, liquid portion and wavy lines inside the cavity). The patient was operated on (pericystectomy) only in May 2022, after several episodes of haemoptysis. Post-operative course was free of complication. ABZ was suspended in October 2022, 6 months after surgery.

**Discussion.** Treatment recommendations for lung CE suggest surgery for active and transitional cysts larger than 5 cm or for complicated cysts of any stage [2,5,6]. Our patient received ABZ while waiting for surgery, eventually requiring more aggressive pericystectomy instead of the commonly performed endocystectomy. While ABZ is not indicated in large pulmonary cysts due to the risk of

rupture, all surgical interventions on the cyst require the use of a one-month course of ABZ as a prophylactic measure [7,8].

**Conclusion.** The forced delay in surgical treatment of the lung cyst caused a shift in cyst stage due to medical treatment (from CE1 to CE4). This is commonly seen in the liver, but rarely in the lung, as lung cysts are normally operated on when they are CE1 or CE3a [9,10].

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## CLASSIFICATION OF EX VIVO LIVER RESECTION AND AUTOTRANSPLANTATION VASCULAR INVASION FEATURES AND ITS ROLE IN PREOPERATIVE EVALUATION AND INTRAOPERATIVE REVASCULARIZATION STRATEGIES

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**Background.** Accurate assessment of vascular invasion in the reserved liver is crucial for ex-vivo liver resection and autotransplantation (ELRA), being helpful to determine whether to implement



ELRA and how to design intraoperative vascular reconstruction. This study aimed to propose a classification of vascular invasion and further elaborate on its significance for preoperative assessment and intraoperative reconstruction.

**Methods.** It falls into a retrospective case series covering 114 patients with hepatic alveolar echinococcosis undergoing ELRA between August 2010 and October 2021, whose characteristics of portal, inferior vena cava, and hepatic venous invasion were retrospectively reviewed and classified. The reconstructive modalities corresponding to vascular typing and the surgical indications of ELRA were in turn proposed tentatively.

**Results.** Portal vein, inferior vena cava, hepatic vein and their collateral circulation are divided into type P0-P3, A; I0-I3, B and H0-H5, C respectively. Corresponding intraoperative vascular reconstruction strategies were proposed based on this classification, and the indications for ELRA were summarized.

**Conclusions.** Classification of vascular invasion may help anatomical understanding, and the corresponding revascularization modality and surgical indications proposed may help surgical decision-making and standardize the approach.

**Key words:** surgical indications ; ex vivo liver resection and autotransplantation ; end-stage hepatic alveolar echinococcosis ; vascular invasion ; classification of vascular.

## NEW CLASSIFICATION OF ALVEOLAR ECHINOCOCCOSIS AS THE BASE OF A NEW STRATEGY REGARDING THE USE OF TRANSPLANT TECHNOLOGIES IN THE LIVER SURGERY

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**Aim of study.** To improve the results of treatment of patients with hepatic alveolar echinococcosis (HAE) by developing and introducing into clinical practice a new classification of the disease, which determines the treatment strategy with all available treatment options, including transplant technologies.

**Material and methods.** A total of 942 patients with HAE in three high-volume centers (1-3) with liver transplantation programs and one non-transplant center (4) were included in the study.

**Results and discussion.** A total of 505 (89.4%) out of 565 patients in the non-transplant center were operated. Radical (R0) liver surgery was possible in 76.5% cases, R1-2 procedures were performed in 19.9% and exploratory laparotomy in 3.6% patients. Radical (R0) hepatectomies and liver transplantation were performed in 366 (97.1%) out of 377 patients by a single protocol in three liver transplant centers (1-3). Patients with HAE were divided into the following groups according proposed new classification of HAE. “Resectable” HAE has a local primary focus, where it is possible to perform traditional liver surgery without vascular reconstruction. In this case, distant metastases that do not determine the severity of the condition may be present, as is moderate fibrosis (F 0–3). In this group there were 99 patients who underwent hepatectomies without vascular reconstruction. “Borderline resectable” HAE has occurred with a spread involving the main vessels of the liver and (or) the inferior vena cava, but there is a sufficient volume of unaffected parenchyma to satisfy metabolic needs. There may be clinically insignificant metastases and minor fibrosis (F 0–2). In borderline-resectable group there were 178 patients. Hepatectomies were performed in combination with vascular reconstructions including cases of normo- or hypothermic total vascular exclusion in vivo or ex vivo. “Non-resectable” HAE is a spread with damage to a critical volume of the



parenchyma, and (or) the invasion of vascular structures, making their reconstruction impossible, and (or) cirrhosis of the FLR in the case of resectable metastases. In 89 patients, the lesion was defining as non-resectable. The non-alternative method of treatment in this group was liver transplantation. “Incurable” HAE: 11 (2.9%) patients with non-resectable metastases which determined the severity of the condition and made no sense of radical liver surgery were considered incurable. Palliative measures were carried out by such patients. Previously performed non-radical interventions in 43.0% of patients were associated with severe complications (Clavien–Dindo III–V) developed after radical hepatectomies and transplantations. Surgical strategy based on the new classification of HAE was effective in 97.1% patients, R0-hepatectomies were performed in 100% cases.

**Conclusion.** Routing patients according to the new classification of hepatic alveolar echinococcosis will improve the treatment results by avoiding non-radical surgery associated with the development of complications after further radical surgical treatment.

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### **МУЛЬТИВИСЦЕРАЛЬНЫЙ ЭХИНОКОККОЗ: ОПРЕДЕЛЕНИЕ, ДИАГНОСТИКА, ЛЕЧЕНИЕ**

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**Актуальность.** Эхинококкоз, вызываемый метацестодами рода *Echinococcus*, является хроническим заболеванием, которое является одним из наиболее распространенных зоонозов во всем мире [1]. При эхинококкозе кисты могут возникать практически в любом анатомическом месте, хотя печень (70–80%) и легкие (10–30%) являются наиболее часто поражаемыми органами, за которыми следуют другие области тела (10–15%) [2]. Хотя описаны случаи эхинококковых кист с множественным поражением органов, нам не удалось найти адекватное определение мультивисцерального эхинококкоза в литературе. Но многие авторы определяют понятие мультивисцерального эхинококкоза как одновременную локализацию эхинококковых кист более чем в одном органе [3]. Случаи мультивисцерального эхинококкоза с атипичной локализацией очень редки, но могут иметь тяжелые последствия и даже привести к летальному исходу. Это серьезная проблема общественного здравоохранения и экономическое бремя, особенно в скотоводческих районах Кыргызской Республики.

**Цель исследования.** Обобщить имеющиеся на сегодняшний день в Национальном хирургическом центре МЗ КР данные по диагностике и лечению мультивисцерального эхинококкоза.

**Материалы и методы.** Исследование проведено в Национальном хирургическом центре МЗ КР, проанализированы данные за 10 лет. За этот период выявлено 84 случая заболевания мультивисцеральным эхинококкозом.

**Результаты.** Распределение по полу показывает, что 38,09% (32 пациента) были женского и 61,9% (52 пациента) мужского пола. Возрастная распространенность эхинококковых кист у пациентов составила от 16 до 80 лет, с пиком заболеваемости у пациентов в возрасте от 16 до 60 лет. Маленький процент встречается у детей и пожилых пациентов. Что касается распределения пациентов по месту жительства, 57 больных (67,58%) проживали в сельской местности, в то время, как только 27 больных (32,14%) проживали в городе. Профессиональная деятельность пациентов важный элемент в эпидемиологии мультивисцерального эхинококкоза. Более высокий процент пациентов с эхинококковыми кистами (60,71%) у животноводов, мясников, ветеринаров, кожевников, охотников, дрессировщиков собак. Распределение больных по вовлеченности органов представлено в табл. 1.

Табл. 1 – Распределение больных по вовлеченности органов

Вовлечение органов	Количество пациентов
правое легкое + печень	20
левое легкое + печень	22
правое легкое + левое легкое	26
правое легкое + левое легкое + печень	9
левое легкое + селезенка	1
правое легкое + головной мозг	1
левое легкое + головной мозг	1
печень + селезенка + поджелудочная железа	1
правое легкое + левое легкое + печень + селезенка	1
правое легкое + левое легкое + головной мозг	1
грудной отдел аорты + печень + селезенка + почки	1

**Обсуждение.** Учитывая относительно частую распространенность мультивисцерального эхинококкоза, обнаружение эхинококкоза в одном органе влечет за собой поиск других локализаций эхинококковых кист, по крайней мере, внутри брюшной и грудной полости. На основании литературных данных и личных находок мы предлагаем алгоритм диагностики мультивисцерального эхинококкоза с поражением грудного и абдоминального полостей: от грудной клетки к животу (алгоритм 1); от живота к грудной клетке (алгоритм 2).

Медикаментозное лечение (химиотерапия) бензимидазолоподобными препаратами имеет показания при мультивисцеральном эхинококкозе периоперационно или между сериями операций. Хирургическое лечение мультивисцеральных очагов может быть одновременным или последовательным (отдельные оперативные вмешательства). Одновременная операция достигается с помощью общего хирургического доступа (если кисты расположены близко друг к другу) или с помощью отдельных хирургических доступов (если кисты расположены в разных органах). В нашем исследовании, одновременная операция через общий хирургический доступ применялся у шести пациентов (справа торакотомия и френотомия справа – 5; левосторонняя торакотомия и френотомия слева – 1). Одновременный доступ через отдельные хирургические разрезы применялся у двух больных (одновременная двусторонняя торакотомия – 1; слева торакотомия и срединная лапаротомия – 1), у 76 больных применялись последовательные хирургические вмешательства (серии операции). Что касается определения порядка оперативных вмешательств при мультивисцеральном эхинококкозе, то он определялся несколькими приоритетами. В первую очередь хирургическое лечение осложненных кист (инфекция, свищ, кровоизлияние) независимо от их локализации, вторым приоритетом являлись неосложненные кисты с локализацией в грудной полости, потому что любая общая анестезия может привести к разрыву эхинококковых кист с вторичным легочным инфицированием. Наконец, третий приоритет — размер кисты, более крупные кисты будут удалены раньше, чем более мелкие.

**Заключение.** Визуализация имеет решающее значение для диагностики мультивисцерального эхинококкоза, особенно в случаях атипичной локализации. По сравнению с моновисцеральным эхинококкозом симптоматика выражена сильнее из-за поражения нескольких органов. Химиотерапия бензимидазолоподобными препаратами имеет хороший эффект при мультивисцеральном эхинококкозе при использовании его периоперационно и между сериями операций. При определении порядка хирургических вмешательств при мультивисцеральном эхинококкозе необходимо учитывать наличие осложнений, локализацию кист в грудной полости и размер кисты.

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## USE OF THE LIGAMENTUM TERES HEPATIS FOR OUTFLOW RECONSTRUCTION DURING EX VIVO LIVER RESECTION AND AUTOTRANSPLANTATION IN PATIENTS WITH HEPATIC ALVEOLAR ECHINOCOCCOSIS: A CASE SERIES OF 24 PATIENTS

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**Background.** Patients with extensive hepatic alveolar echinococcosis might require ex vivo liver resection and autotransplantation to remove the lesion. Patients with extensive vascular invasion will need reconstruction, but the selection of the proper graft is complicated. This study aimed to investigate the effectiveness and adverse events of using the ligamentum teres hepatis as a vascular replacement graft in ex vivo liver resection and autotransplantation.

**Methods.** This was a retrospective case series of patients with hepatic alveolar echinococcosis who underwent ex vivo liver resection and autotransplantation between August 2010 and October 2018 and in whom the ligamentum teres hepatis was used to repair the remnant liver. The operative outcomes, recurrence, and survival were examined.

**Results.** Twenty-four patients were included (10 men, 14 women). The anhepatic period was 290 to 672 minutes (median of 450 minutes). The ratio of the remnant liver volume to the standard liver

volume was 0.43 to 0.97 (median of 0.71). The blood loss was 1,000 (500e5,000) mL. The postoperative hospital stay was 23 (1e85) days. Of the 24 patients, 3 died after the operation, but those deaths were unrelated to liver vascular complications.

**Conclusion.** The ligamentum teres hepatis could be used as a vascular replacement graft in ex vivo liver resection and autotransplantation. It has the advantages of convenient specimen extraction, no donor site injury, and no immunological rejection, which has promising clinical application prospects.

**Key words:** ligamentum teres hepatis, ex vivo liver resection, autotransplantation.

## COLLATERAL CIRCULATION CAUSED BY END-STAGE HEPATIC ALVEOLAR ECHINOCOCCOSIS

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**Background.** Hepatic *alveolar echinococcosis* (HAE), as a benign parasitic disease with malignant infiltrative activity, grows slowly in the liver, allowing sufficient time for collateral vessels to emerge in the process of vascular occlusion.

**Methods.** The portal vein (PV), hepatic vein and hepatic artery were observed by enhanced CT and the inferior vena cava (IVC) by angiography, respectively. Analysis of the anatomical characteristics of the collateral vessels helped to look into the pattern and characteristics of vascular collateralization caused by this specific etiology.

**Results:** 33, 5, 12 and 1 patients were included in the formation of collateral vessels in PV, hepatic vein, IVC and hepatic artery, respectively. PV collateral vessels were divided into two categories according to different pathways: type I: portal-portal venous pathway (13 cases) and type II: type I incorporates a portal-systemic circulation pathway (20 cases). Hepatic vein (HV) collateral vessels fell into short hepatic veins. The patients with IVC collateral presented with both vertebral and lumbar venous varices. Hepatic artery collateral vessels emanating from the celiac trunk maintains blood supply to the healthy side of the liver.

**Conclusions.** Due to its special biological nature, HAE exhibited unique collateral vessels that were rarely seen in other diseases. An in-depth study would be of great help to improve our understanding related to the process of collateral vessel formation due to intrahepatic lesions and its comorbidity, in addition to providing new ideas for the surgical treatment of end-stage HAE.

**Key words:** collateral circulation, hepatic *alveolar echinococcosis*, end-stage, portal vein cavernous degeneration.

## RECONSTRUCTION OF HEPATIC VENOUS OUTFLOW AND MANAGEMENT OF ITS COMPLICATIONS USING EX VIVO LIVER RESECTION AND AUTOTRANSPLANTATION: A SINGLE-CENTER EXPERIENCE

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**Background.** This study was designed to investigate the techniques of hepatic venous outflow reconstruction and the management of its complications using *ex vivo* liver resection and autotransplantation (ELRA).

**Methods.** Being a retrospective case series covering 84 patients who underwent hepatic venous outflow reconstruction during ELRA from January 2016 to October 2020, 11 cases of postoperative hepatic venous outflow obstruction (HVOO), whose surgery details were described and survival rates analyzed.

**Results.** A total of the 84 alveolar Echinococcosis (AE) series was no intraoperative death. The 30-day mortality was 5.95% (5 /84). The most common postoperative complication was pleural effusion in 21 cases (25%). HVOO occurred in 11 cases, one of them died of liver failure, and the other 10 patients underwent interventional revascularization with good results. There was no significant difference in survival between patients with successful interventional revascularization due to HVOO and patients without HVOO ( $P > 0.05$ ).

**Conclusions.** Individualized and well design reconstruction of hepatic vein can be considered as a key procedure to reduce the complications of HVOO in ELRA. Once HVOO occurs, emergent management must be performed immediately before liver dysfunction. Interventional revascularization showed an effective approach, though the more clinical cases need to be evaluated.

**Key words:** complications, *ex vivo* liver resection and autotransplantation, hepatic venous outflow, outflow obstruction, transplantation.

## RECONSTRUCTION OF INFERIOR VENA CAVA IN *EX-VIVO* LIVER RESECTION AND AUTO-TRANSPLANTATION

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**Background.** Hepatic alveolar echinococcosis (AE) is most commonly found in retrohepatic inferior vena cava (RHIVC). *Ex-vivo* liver resection and auto-transplantation (ELRA) can better realize the radical resection of end-stage hepatic AE with severely compromised hepato-caval confluences, and the reconstruction of the affected vessels. Currently, there is a scarcity of information regarding RHIVC reconstruction in ELRA.

**Objective.** To propose reasonable RHICV reconstruction strategies for *ex-vivo* liver resection and auto-transplantation.

**Methods.** We retrospectively summarized the clinical data of 114 patients diagnosed with hepatic AE who treated by ELRA in our department. Totally 114 patients were divided into the three groups according to the different reconstruction methods of RHIVC, including group A with original RHIVC being repaired and reconstructed ( $n = 64$ ), group B with RHIVC being replaced ( $n = 43$ ), and group C with RHIVC being resected without reconstruction ( $n = 7$ ). The clinical data of patients, including the operation time, anhepatic phase, intraoperative blood loss, complications and postoperative hospital stay, were analyzed and the patients were routinely followed up. The normally distributed continuous variables were expressed as means  $\pm$  SD, whereas the abnormally distributed ones were expressed as median and analyzed by ANOVA. Survival curve was plotted by the Kaplan–Meier method.



**Results.** All patients were routinely followed up for a median duration of 52 (range, 12-125) months. The 30 d mortality rate was 7.0% (8/114) and 7 patients died within 90 d. Among all the subjects, the inferior vena cava (IVC)-related complication rates were 17.5% (11/63) in group A and 16.3% (7/43) in group B. IVC stenosis was found in 12 patients (10.5%), while thrombus was formed in 6 patients (5.3%). Twenty-two patients had grade III or higher complications, with the complication rates being 17.2%, 16.3% and 57.1%, in the three groups, respectively. The average operation time was  $16.7 \pm 2.9$  h for group A,  $15.5 \pm 3.1$  h for group B and  $16.9 \pm 4.1$  h for group C, and the average anhepatic phase was  $418.4 \pm 108.3$  min,  $383.9 \pm 117.0$  min, and  $337.4 \pm 108.7$  min in groups A, B and C. The average postoperative hospital stay in the three groups was  $32.3 \pm 19.8$  d,  $26.7 \pm 18.2$  d, and  $51.3 \pm 29.4$  d ( $P = 0.03$ ), respectively. The survival rates of group A and group B were quite close, while that of group C was lower, but the differences were not statistically significant ( $P = 0.81$ ).

**Conclusion:** ELRA can be considered as a safe and feasible option for end-stage hepatic AE patients with RHIVC infiltration. The RHIVC reconstruction methods should be selected appropriately depending on the defect degree of AE lesions in IVC lumen. The RHIVC resection without any reconstruction method should be considered with caution.

**Key Words:** *alveolar echinococcosis, ex-vivo liver resection, liver transplantation, inferior vena cava.*

## HLA-POLYMORPHISMS INFLUENCING DISEASE CHARACTERISTICS AND OUTCOME IN PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS

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**Relevance.** This study aims to identify HLA-haplotypes which predispose to extensive disease at first presentation (PNM stage IV) as well as a progressive and stable course of disease respectively.

**Methods.** We performed a case-control-study analysing the HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 -polymorphisms of 173 patients with probable or confirmed AE and a follow-up of at least 2 years.

**Results.** Patients with HLA-C6 had lower odds (OR=0.31, 95%CI=0.12-0.79,  $p=0.010$ ) for an extensive disease and patients with HLA-A1 had higher odds for a large lesion ( $\geq 100\text{mm}$ ) at first presentation (OR=3.0, 95%CI=1.3-6.9,  $p=0.008$ ). Patients with HLA-DRB1\*7 were more likely to develop disease progression (OR=2.99, 95%CI 1.12-7.98,  $p=0.024$ ). On the other hand, HLA-A2 increased the odds of stable disease without needing medical treatment (OR=3.07, 95%CI=1.07-8.83,  $p=0.031$ ).

**Discussion.** These results indicate that HLA-polymorphisms influence the course of disease in AE patients. Previously, Godot et al. [1] reported HLA B8, DR3, DQ2 and Eiermann et al. [2] HLA-DQB1\*02 to be associated with more severe forms of AE.

## HEPATIC MACROPHAGES: KEY PLAYERS IN THE ESTABLISHMENT AND GROWTH OF HYDATID CYSTS IN THE LIVER DURING ECHINOCOCCUS GRANULOSUS SENSU STRICTO INFECTION

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**Relevance.** *Echinococcus granulosus sensu lato* (*E. granulosus s.l.*) larvae, which form hydatid cysts, are the causative agent of cystic echinococcosis (CE). The larvae predominantly dwell in the liver and grow like a unilocular bladder that is usually characterized by mild local inflammation [1,2]. Macrophages constitute the main cellular component of the liver and play a central role in controlling the progression of inflammation and liver fibrosis [3]. However, the role of hepatic macrophages in the establishment and chronicity of infection with *E. granulosus s.l.* larvae has not been fully elucidated.

**Materials and methods.** We investigated the heterogeneity characteristics and functions of hepatic macrophages in CE patients and *Echinococcus granulosus sensu stricto* (*E. granulosus s.s.*)-infected mice at different infection stages.

**Results.** We showed that hepatic macrophages predominantly with an anti-inflammatory phenotype were involved in the tight control of liver inflammation in both CE patients with active

cysts and *E. granulosus* s.s.-infected mice at the late stage to favor persistent infection. Notably, we found that the pool of hepatic macrophages expanded dramatically by attracting massive amounts of monocyte-derived macrophages (CD11b<sup>hi</sup> F4/80<sup>int</sup> MoMFs) to the infection site that preferentially polarized toward a proinflammatory phenotype (iNOS<sup>+</sup>) to eliminate parasites at the early stage of infection and then polarized toward an anti-inflammatory phenotype (CD206<sup>+</sup>) to favor persistent infection at the later stage. Depletion of hepatic macrophages promoted *E. granulosus* s.s. larvae establishment and growth in the liver by inhibiting CD4<sup>+</sup> T cell infiltration and liver fibrosis in the mouse model.

**Conclusion.** These findings demonstrate that hepatic macrophages play a vital role in the pathogenesis of CE, contributing to a better understanding of the local inflammatory responses in CE and possibly facilitating the design of novel therapeutic approaches for CE by targeting hepatic macrophages.

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## UPREGULATION OF LAG3 MODULATES THE IMMUNE IMBALANCE OF CD4<sup>+</sup> T-CELL SUBSETS AND EXACERBATES DISEASE PROGRESSION IN PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS AND A MOUSE MODEL

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**Relevance.** Infection with the cestode *Echinococcus multilocularis* (*E. multilocularis*) causes alveolar echinococcosis (AE), a tumor-like disease predominantly affecting the liver but able to spread to any organ<sup>[1]</sup>. T cells develop functional defects during chronic *E. multilocularis* infection, mostly due to upregulation of inhibitory receptors such as T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT) and programmed death-1 (PD-1)<sup>[2]</sup>. However, the role of lymphocyte activation gene-3 (LAG3), an inhibitory receptor, in AE infection remains to be determined.

**Materials, methods and Results.** Here, we discovered that high expression of LAG3 was mainly found in CD4<sup>+</sup> T cells and induced regulatory T cells (iTregs) in close liver tissue (CLT) from AE patients. In a mouse model of *E. multilocularis* infection, LAG3 expression was predominantly found in T helper 2 (Th2) and Treg subsets, which secreted significantly more IL-4 and IL-10, resulting in host immune tolerance and disease progression at a late stage. Furthermore, LAG3 deficiency was

found to drive the development of effector memory CD4<sup>+</sup> T cells and enhance the type 1 CD4<sup>+</sup> T-cell immune response, thus inhibiting metacestode growth *in vivo*. In addition, CD4<sup>+</sup> T cells from LAG3-deficient mice produced more IFN- $\gamma$  and less IL-4 when stimulated by *E. multilocularis* protoscoleces (EmP) antigen *in vitro*. Finally, adoptive transfer experiments showed that LAG3-knockout (KO) CD4<sup>+</sup> T cells were more likely to develop into Th1 cells and less likely to develop into Tregs in recipient mice.

**Conclusion.** Our work reveals that high expression of LAG3 accelerates AE disease progression by modulating the immune imbalance of CD4<sup>+</sup> T-cell subsets. These findings may provide a novel immunotherapeutic strategy against *E. multilocularis* infection.

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## EFFECTS OF MANAGEMENT OF INFECTION SOURCE OF ECHINOCOCCOSIS, XINJIANG UYGUR AUTONOMOUS REGION OF CHINA

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**Background.** Echinococcosis is highly endemic in western and northern China. Xinjiang Uygur Autonomous Region is one of the most serious prevalent area. Dogs are the primary infection source for the transmission of echinococcosis to humans. A control and prevention campaign based on dog management has been implemented in 2016. This study aims to evaluate the effects of dog management on the infection rate of dogs.

**Methods.** Data of dog population, registration and de-worming of 81 hydatid counties in Xinjiang Uygur Autonomous Region between 2016 and 2022 were obtained from the annual prevention and control report. Domestic dog fecal samples were collected from 81 hydatid counties in Xinjiang Uygur Autonomous Region in 2016 to determine the infection of domestic dogs using coproantigen enzyme-linked immunosorbent assay (ELISA). Data analysis was processed using SPSS statistics to compare dog infection rate between 2016 and 2022 by chi-square test.

**Results.** In Xinjiang Uygur Autonomous Region domestic dog population has decreased from 511500 in 2016 to 313900 in 2022, while the registration rate has increased from 85% in 2016 to 98.3% in 2022. Similarly, Dog de-worming frequency has increased from 10 times per annum in 2016 to 11 times in 2022, indicating that approximately every dog was dewormed 34 day. A total of 112800 dog fecal samples were collected for coproantigen ELISA assay. The dog infection rate was 2.02% (649/32055) in 2016, which was significantly higher than 0.18% (147/80811) in 2022 ( $P < 0.05$ ).

**Conclusions.** Increased dog registration, decreased dog population, and increased dog de-worming frequency contributed to significantly decrease the dog infection rate in Xinjiang Uygur Autonomous Region. Control and prevention campaign based on dog management could significantly decrease dog infection with *Echinococcus* spp. in echinococcosis endemic areas.

**Key words:** Echinococcosis, dog management, dog infection, Xinjiang Uygur Autonomous Region, China

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## COMMITMENT TO THE CONCEPT OF COMMON HEALTH IN SURVEILLANCE OF ECHINOCOCCOSIS IN KAZAKHSTAN

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The report will reflect retrospectively information on the epidemiological situation of echinococcosis and infection rates of *Echinococcus* spp. among the population of Kazakhstan; granularity will be given in the context of administrative territories, age and other epidemiological characteristics, the state of the affected population at the current moment. The key principles of



common health in the supervision of echinococcosis in the country, the interaction of medical and non-medical organizations, the principles of preventive and anti-epidemic measures among decreed population groups, methodological approaches to ensuring infectious safety during operations to remove echinococcosis cysts will be highlighted. The results obtained are reflected in the master's thesis for the specialty "Medical and preventive work".

## **EFFECTIVENESS OF ECHINOCOCCOSIS CONTROL ON EAST QINGHAI-TIBET PLATEAU OF SICHUAN PROVINCE, CHINA: AN CRITICAL ASSESSMENT AFTER 15 YEARS OF INTERVENTION**

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**Relevance** Echinococcosis is a major zoonosis of public health significance in west China. A national control programme was initiated in 2006 in 10 counties on east Qinghai-Tibet plateau of Sichuan Province, where the disease was prevalent. Totally 35 counties, where the population was some 3 million, were included to the programme since 2012. The programme began with dog deworming with praziquantel and surveillance, gradually it included health education, purchasing of lungs and livers of livestock slaughtered, reduction of stray dogs, elimination of small mammals around settlements, and most recently vaccination of sheep or goat were brought into the programme.

**Materials And Methods** Surveillance and human prevalence data from 2006 to 2021 were accessed from Sichuan Provincial Center for Disease Prevention and Control. Dog Echinococcus spp. infections were detected by copro-Elisa; human prevalence were obtained by yearly mass human ultrasound screening with serology test confirmation according to WHO recommendations; livestock cystic echinococcosis prevalence and small mammals alveolar echinococcosis prevalence were calculate based on necropsy of livers and lungs, and confirmed with PCR. Descriptive and inference statistical methods were used to analyze the data.

**Results** After many years of control, incident rate of human cases, dog infection rate, and livestock disease rate decreased from 0.30%, 15.88%, and 8.05% in 2010 to 0.02%, 0.46%, and 1.07% in 2022, respectively. However, there were some 60 new reported patients every year in the past 2 years, which indicated ongoing transmission of the disease.

**Conclusion** It seems that the programme does make some progress, however, further researches should be done to understand the reasons behind the ongoing transmission.

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**NEGLECTED YET PERVASIVE: SHEDDING LIGHT ON ECHINOCOCCOSIS  
AWARENESS IN KYRGYZSTAN AND PREVENTION CAPACITY  
VIA CROSS-SECTIONAL STUDY FINDINGS**

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**Introduction.** Human cystic echinococcosis (CE) continues to present a significant public health challenge in resource-restricted areas, particularly in Central Asia. Our study focused on assessing disease awareness and provided information for evidence-based policies in future prevention efforts in a highly endemic CE country, Kyrgyzstan(KR).

**Methods.** A cross-sectional study was conducted with 242 participants from Bishkek and Issyk-Kul oblast and utilized survey data to analyze demographics, household information, CE-related practices, and CE knowledge. Using propensity scores identified participants in high-risk environments (HRE) and engaging in high-risk behaviors (HRB) linked to CE contracting.

**Results.** Out of 242 participants, 97 (40%) lived in HRE, with 53 (54.6%) engaging in HRB linked to CE. Only 14 (6%) followed all preventive measures, while 136 (56%) followed some, and 106 (44%) practiced HRB. 237(97.5%) of participants had heard about CE, but only 14 (6%) identified all transmission routes, and 154(63.4%) were unaware of dog contact as a route. Education reduced HRB risk (OR=0.5, 95% CI=0.23, 0.80).

**Conclusion.** The study's main findings are alarming. Most participants lacked complete knowledge about the disease and engaged in risky behavior. Urgent prevention programs targeting CE awareness in the KR population are crucial to addressing challenges posed by nomadic habits within the population.

**VACCINATION AGAINST CYSTIC ECHINOCOCCOSIS:  
PRODUCTION OF THE EG95 VACCINE AS A BACTERIN**

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The EG95 vaccine provides a high level of protection against cystic echinococcosis in animal hosts. The vaccine is now manufactured and is a licensed product in China (Chongqing Auleon Biologicals), Argentina (Tecnovax) and Morocco (MCI Sante Animale) and is also registered for use in several other countries. The vaccine is in practical use in China and in Argentina. The current commercial vaccine is produced from *Escherichia coli* containing a plasmid which directs expression of the EG95 protein. Following fermentation the bacteria expressing EG95 are disrupted and centrifuged. Much of the EG95 protein is contained in insoluble inclusion bodies. These are processed and finally solubilized using urea. The process requires manipulation of the product in order to complete the isolation of the vaccine components.

A slight modification to the cDNA encoding the EG95 protein was found to lead to a substantial increase in soluble EG95 in bacterial lysates. We tested the clarified lysate as a crude bacterin vaccine

[1] in thirty merino-cross lambs. Vaccinated animals received 2 subcutaneous immunizations 4 weeks apart in the neck with vaccines comprising 1mg Quil A adjuvant (Brenntag Biosector A/D, Denmark) together with either 50 µg affinity purified EG95NC<sup>-</sup> protein or the same quantity of EG95NC<sup>-</sup> as part of the total soluble *E. coli* protein (bacterin). The control group received no vaccination. Two weeks after the second vaccinations, each lamb was given an intraruminal infection with 1000 eggs of *E. granulosus*. Approximately 1 year after the challenge infection, the liver and lungs were sliced by hand at approximately 3–4mm intervals to identify *E. granulosus* cysts.

Both affinity purified EG95NC<sup>-</sup> and non-purified EG95NC<sup>-</sup> (consisting of total soluble bacterial proteins) induced high levels of protection (94.6 and 97.9%, respectively) in comparison to controls, with no significant difference between the two EG95NC<sup>-</sup> groups (Mann–Whitney U test). All control animals developed large numbers of viable cysts following the challenge infection, ranging from 195 to 503 cysts per animal.

The EG95 vaccine produced as either affinity purified antigen, or as total soluble protein from *E. coli*, induced very high levels of protection against a challenge infection with *E. granulosus*. The presence of contaminating bacterial proteins (up to ~50%) did not affect the ability of the EG95NC<sup>-</sup> antigen to protect vaccinated sheep against *E. granulosus* infection. Based on the bacterial fermentation conditions used, 1 litre of bacterial culture expressing EG95NC<sup>-</sup> would produce more than 7000 doses of vaccine.

Many of the regions where cystic echinococcosis is highly endemic are in relatively poor, developing countries emphasizing the need to minimize vaccination costs. Widespread implementation of vaccination against *E. granulosus* in livestock has the potential to reduce the incidence of human cystic echinococcosis in endemic areas. Our demonstration of a simple, comparatively inexpensive method for producing the EG95 vaccine may facilitate the vaccine's production in more countries where cystic echinococcosis is highly endemic, contributing to the parasite's control and a reduction in human infections.

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## EVALUATION OF A PLASMID VACCINE ENCODING IMMUNOGENIC EPITOPES OF *ECHINOCOCCUS GRANULOSUS* AGAINST CYSTIC ECHINOCOCCOSIS

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**Background.** The present study was conducted to design and evaluate a plasmid vaccine encoding *Echinococcus granulosus* Eg95-1 to EG95-6, P29 and GST against cystic echinococcosis in BALB/c mice.

**Materials and Methods.** At first, a multi-epitope vaccine was designed. Then it was transformed into *Escherichia coli* TOP10 by recombinant pcDNA plasmid 3.1. Then the bacteria were mass produced and the desired plasmid was extracted. BALB/c mice were immunized with concentrations of 50 and 100 µg of plasmid together with IL-12 adjuvant or alone.

**Results.** The mice receiving doses of plasmid, especially the concentration of 100 µg, alone or with adjuvant, created Th1 type immunity, so that they created higher levels of IgG2a and IFN-γ antibodies in the mice, on the contrary, the mice showed lower levels of IL-4. After challenge, hydatid cysts were not observed in most of the vaccinated groups, while numerous cysts were developed in the mice of the control group.

**Conclusion.** Our results highlighted the adequate immunogenic potential of this vaccine candidate.

## ULTRASONOGRAPHY SCREENING OF HEPATIC CYSTIC ECHINOCOCCOSIS IN SHEEP FLOCKS USED FOR EVALUATING CONTROL PROGRESS IN A REMOTE MOUNTAIN AREA

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**Background.** Although ultrasonography (US) has been widely used in diagnosing human diseases, it needs to be adjusted for screening hepatic cystic echinococcosis (CE) in sheep flocks for monitoring control progress. In this study, we used a US scanner to screen sheep flocks and evaluated the control efficacy of dosing dogs once a year with praziquantel for 7 years from 2014–2021.

**Methods.** All sheep in the three flocks were screened using the book model US scanner used in 2014 and compared with the prevalence in 2021 in Bayinbuluke in Xinjiang, China. Sheep age was determined using their incisor teeth. The activity and fertility of cysts were determined using a US image. Deworming dogs with praziquantel once a year was the technique for controlling echinococcosis in the community.

**Result.** Three flocks had 968 sheep in 2014, with 13.22%, 22.62%, 18.7%, 27.27%, 11.88%, and 6.3% of the sheep aged 1, 2, 3, 4, 5, and  $\geq 6$  years old, respectively. US scanning revealed that the overall CE prevalence was 37.40% (362/968) with 9.40% (91/968) and 28.00% (271/968) of the sheep harboring active cysts and calcified cysts, respectively. There was no significant difference in infection rates of sheep between 2014 and 2021 ( $P > 0.05$ ). The one book-model US scanner could detect 100–150 sheep per day.

**Conclusions.** US is a practical tool for field screening of CE in sheep flocks. Old sheep play a key role in the transmission of CE, especially culled aged sheep. Dosing dogs once a year has no effect on the control of echinococcosis.

**Key words:** ultrasonography; cystic echinococcosis; *Echinococcus granulosus*; sheep flocks; fertile cysts.

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## PREVENTION, DIAGNOSIS AND MANAGEMENT OF ECHINOCOCCOSIS IN CHINA

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**Background.** *Echinococcosis* is a zoonosis caused by cestodes of the genus *Echinococcus* (family Taeniidae). This serious and near-cosmopolitan disease continues to be a significant public health issue, with western China being the area of highest endemicity for both the cystic (CE) and alveolar (AE) forms of *echinococcosis*. *Echinococcosis* is endemic in 12 provinces, accounting for 44% of the total land area of China, and 7 provinces or autonomous regions in the northwest are endemic, resulting in an annual direct economic loss of 3 billion yuan.

**Methods.** The long-term sustained efforts have been made for the prevention, diagnosis and management of echinococcosis in China. The special networks, designated hospitals and tele-medicine have been practiced to promote standardized protocol for prevention, diagnosis and management of echinococcosis.

**Results.** During 2005-2006, human echinococcosis prevention, control and treatment network initially has been setup in Xinjiang. The network included 29 hospitals in 2008 and carried out 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. Tele-Medicine center was established at author institution in 16th April, 2008, and played crucial role in personnel training, prevention, control and treatment of echinococcosis. 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. The standardized protocol of preoperative imaging assessment including ultrasound, CT, MRI and PET-CT scan have been promoted around the country and the diagnosis has been greatly improved. Multidisciplinary team discussion is mandated for the clinical management of echinococcosis. Total cystectomy of CE has been advocated around country and the post-operative recurrence and morbidity have been greatly decreased. Innovative practice of ex-vivo liver resection and autotransplantation has been pioneered in AE management with nearly 140 cases with promising results.

**Conclusion.** Over the decades, the prevention, diagnosis and management of echinococcosis have been greatly improved.

**Key words:** *alveolar echinococcosis, cystic echinococcosis, Prevention, Diagnosis, Treatment, Zoonosis.*

## CONTROL STRATEGY IN THE COMMUNITIES WITH BOTH ALVEOLAR ECHINOCOCCOSIS AND CYSTIC ECHINOCOCCOSIS HIGHLY ENDEMIC IN WESTERN CHINA

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**Relevance.** Both alveolar echinococcosis (AE) and cystic echinococcosis (CE) are highly endemic in western China. For stopping the transmission of both pathogens in communities in this area, we deworm dogs with baited praziquantel every month without special meat inspection for slaughtered animals in villages.



**Materials and methods.** To effectively and enduringly carry out the dosing dogs with high dosing frequency of dosing dogs in villages, a special administration leading group was established in a county level and each village had a control officer for feeding dogs with baited praziquantel. Purging method or copro-ELISA was used for monitoring dog infection with Echinococcus.

**Results.** For about 20 years from 1996 to 2016 of control, both AE and CE human cases were dropped to 0.07% from 2.63%. The prevalence of CE in sheep was dropped to 2.53% from 42.28% and the prevalence of Echinococcus spp in dogs was dropped to 1.53% from 11.30%. In the national level, both AE and CE were also significantly decreased.

**Conclusion.** Given both AE and CE are highly endemic in western China, monthly dosing dogs with baited praziquantel plays a key role in control of both these two echinococcoses in these areas.

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## EPIDEMIOLOGICAL CHARACTERISTICS OF ECHINOCOCCOSIS IN YILI PREFECTURE OF XINJIANG FROM 2005 TO 2020

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**Objective.** In order to understand the epidemiologic characteristics and status of echinococcosis in Yili Prefecture of Xinjiang Uygur Autonomous Region from 2005 to 2020.

**Methods.** Data from Echinococcus cases in Yili Prefecture of Xinjiang during 2005 and 2020 were collected from National Infectious Disease Reporting Information Management System and analyzed using descriptive epidemiology approaches.

**Results.** The results showed that 4612 echinococcosis cases were reported in Yili Prefecture from 2005 to 2020. The average annual incidence rate was 9.92/100 000. The number of reported cases peaked in 2017, followed by a decline annually, with no significant seasonal patterns. Cases were reported in all 11 counties (cities) of Yili Prefecture. Chabuchaerxibo Autonomous County (20.96/100 000), Zhaosu County (19.94/100 000) and Nileke County (16.61/100 000) had the highest incidence rate. Of all the reported cases, 2 488 cases were male, with an average annual incidence of 10.46/100 000; 2 124 cases were female, with an average annual incidence of 9.27/100 000. There was no significant difference in annual incidence between males and females ( $\chi^2 = 1.063$ ,  $P > 0.05$ ). The average age of the reported echinococcosis patients was 39 years, while the majority of the cases were aged between 20 and 59, accounting for 75.1% (3 462 cases) of the total cases. The occupation was mainly farmers and herdsmen, accounting for 63.1% (2 909 cases) of the cases.

**Conclusions.** It is suggested that the prevalence of echinococcosis in Yili Prefecture of Xinjiang is high, affecting a large number of people suggesting the need for developing science-based and effective prevention approaches that are suitable for controlling the transmission of echinococcosis.

# A PILOT STUDY OF ECHINOCOCCOSIS CONTROL BY MONTHLY DEWORMING DOGS USING AN INTERNET BASED DRUG DELIVERY SYSTEMS IN TIBETAN VILLAGES

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**Relevance.** In Qinghai-Tibetan plateau both alveolar echinococcosis (by *Echinococcus multilocularis*) and cystic echinococcosis (by *E. granulosus*) are highly endemic. Monthly dosing dogs with praziquantel (PZQ) by an authorized villager has been used as a technical method for many years and showed much progress. However, it is still difficult for hiring a villager to deliver the deworming drug to every family every month, especially to these in the nomadic communities.

**Materials and methods.** To efficiently feed the dog with PZQ in nomadic sheep farms, we established an internet system containing an electronic box containing 36 baited PZQ tablets and a speaker to monthly remind villagers to deworm their dogs. The system also has a daily sound education program on prevention, diagnosis, treatment and control of echinococcosis. For grain-growing villages, two vending drug machines were placed for automatically delivering PZQ medicine once a villager receives dosing dog message from the system through mobile system. PZQ tablets were released from the machines once villagers tip the electronic key to the machine after registration. The villagers had a Wechat group for sending pictures of feeding their dogs through mobile once the dogs took the medicine. Every dog dosing was recorded by the system and automatically generated a report monthly.

**Results.** Dosing dog rates of each month was 89-78% in Tibetan nomadic villages. In grain-growing villages, the dosing dog rate of each month was 100-84%. The prevalence of *E. granulosus* in dogs (copro-ELISA) were dropped from 6.25% to 0.0% and the infectious rate of sheep dropped from 17.3% to 2.4%.

**Conclusion.** The internet based drug delivery systems are efficient for controlling both alveolar echinococcosis and cystic echinococcosis by reminding and encouraging villagers to deworm their dogs monthly.

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## ENVIRONMENTAL DISTRIBUTION OF CONTAMINATED DOGS FEACES IN KYRGYZSTAN

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**Abstract.** The results of the study showed that no stable correlation was established between cases of human infection with alveococcosis and echinococcosis and detected positive samples of dog feces. A comparative study in Kochkor district of dog faecal samples was conducted (between 2008 and 2017), the result showed that there was no difference in prevalence of *E. granulosus s.l.* (2.8%), *E. multilocularis* (3.4%) and *Taenia spp.* (16.3%). An increase in the contamination of eggs of parasites in the regions occurs in the autumn, after the return of farmers with dogs from summer mountain pastures.

## MOLECULAR CHARACTERIZATION OF CATTLE AND SHEEP ISOLATES OF ECHINOCOCCUS GRANULOSUS FROM ELAZIG PROVINCE IN TURKEY AND EXPRESSION ANALYSIS OF THE NON-CODING RNAS, EGR-MIR-7, EGR-MIR-71 AND EGR-MIR-96

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Cystic Echinococcosis (CE) is a common zoonotic disease seen in human and animals worldwide, caused by the larval form of *Echinococcus granulosus*. In this study, *E. granulosus s.l.* species and haplotypes were determined in hydatid cysts isolated from cattle and sheep, and the expression levels of egr-miR-7, egr-miR-71 and egr-miR-96 miRNAs were compared in different cyst structures. A total of 82 (cattle, n=41; sheep, n=41) hydatid cyst isolates (germinal membranes and/or protoscoleces) were collected from a slaughterhouse in Elazig province of Turkey. After mt-CO1 gene sequences were made, 81 out of 82 hydatid cyst isolates were determined as *E. granulosus s.s.* (G1 and G3), while an isolate of cattle origin was determined as *Echinococcus canadensis* (G6/7). A total of 26 nucleotide polymorphisms and 29 haplotype groups were identified in the samples. miRNA expressions in germinal membranes of sterile cysts and germinal membrane and protoscoleces of fertile cysts were investigated by qRT-PCR and Real Time PCR analyses. It was determined that miRNAs were expressed at high levels in 79.31% of the 29 haplotype groups and at low levels in the remaining 10.34%. In 10 fertile samples of sheep origin, egr-miR-7, egr-miR-71 and egr-miR-96 miRNAs were found to be 44, 168, and 351-fold higher in expression, respectively, in the germinal membrane compared to the protoscoleces. Especially egr-miR-96 may have the potential to be used as biomarkers in the diagnosis of active CE.

## SYLVATIC LIFECYCLES OF ECHINOCOCCUS SPP. IN MAMMALS OF SUB-SAHARAN AFRICA

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**Relevance.** Several *Echinococcus* species, such as *E. granulosus* s.s., *E. canadensis* G6/7, *E. equinus*, *E. ortleppi* and *E. felidis* are endemic to sub-Saharan Africa and have been recorded from various herbivore and carnivore hosts. However, the majority of the *Echinococcus* cases previously reported from African wildlife remain unspecified, as molecular methods for identification weren't available at the time. For most regions, recent information on the distribution and prevalence of *Echinococcus* in wildlife species is scarce.

While the Cystic Echinococcosis in sub-Saharan Africa Research Initiative (CESSARi) primarily aims to gather information on the epidemiology of CE within humans and domestic animals, another focus of the project lies on gaining insights on the involvement of sylvatic lifecycles in transmission dynamics and the identification of suitable wildlife hosts for *Echinococcus* species.

**Materials and methods.** The CESSARi project, which is funded by the German Research Foundation (DFG), involves medical, veterinary and biological research institutions in Ethiopia, Ghana, Kenya, Namibia, Zambia, Germany and associated researchers from other countries. In order to assess the importance of different wildlife species as reservoirs and transmitters of *Echinococcus* and their role in spill-over events, various carnivores and herbivores were investigated. Cestode material obtained through necropsies and faecal samples were collected from selected study sites and subjected to molecular identification.

**Results.** At wildlife-livestock interfaces, lifecycles of *E. granulosus* s.s., *E. canadensis* G6/G7 and *E. ortleppi* were found to involve both wild and domestic hosts. Lions were identified as suitable hosts for most, if not all, CE agents.

**Conclusion.** The recent wildlife data collected by CESSARi give further understandings of the importance of conservation strategies and livestock management, in dealing with *Echinococcus* spill-overs between wild and domestic animals. Related future studies at different study sites will help ascertain further epidemiological information.

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## THE CHANGING FACE OF ECHINOCOCCUS GRANULOSUS TRANSMISSION IN AUSTRALIA AND THE IMPACT OF HYDATID INFECTION ON CATTLE CARCASS WEIGHT

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The life cycle of *Echinococcus granulosus* in Australia consists of a domestic pattern of transmission involving domestic dogs and livestock and a sylvatic pattern involving dingoes and macropodid marsupials, particularly a range of wallaby species. Hydatid disease was a major public health issue in many parts of Australia from shortly after settlement until recently. The only official hydatid control program in Australia was on the island of Tasmania from 1962-1996 and effectively eliminated the parasite from the island. On mainland Australia there has never been an official hydatid control program, but there have been a series of small local unofficial programs. *Echinococcus granulosus* infection was common in Australian rural dogs because, until the development of praziquantel in the mid 1970's, there was no effective chemical treatment against *E. granulosus* infection in dogs and farmers also commonly fed their dogs with sheep livers and lungs.

Praziquantel is now 'off-patent' and included in generic, inexpensive deworming products for dogs, also commercial, cheap, highly palatable, dry dog food is widely available and education programs by state departments of agriculture regarding the danger of feeding offal to dogs have been ongoing for a number of years. As a result, hydatid infection in humans in Australia is now rare. The occurrence of *E. granulosus* infection in dogs and sheep is now also uncommon compared to 20-30 years ago. However, the wildlife reservoir for transmission of *E. granulosus* remains large and widespread. Most infections that occur in domestic livestock occurs where there is interaction of livestock with dingoes.

This is particularly the case in cattle because in eastern Australia, cattle commonly graze in areas where the pasture is unsuitable for sheep and there is a high population of dingoes that would predate on sheep. A recent study on the impact of hydatid infection in over 1.6 million cattle has shown the prevalence of infection to be commonly around 30% but in some areas the prevalence was as high as 70%. Most cysts occurred in the liver (94%) whilst 44% of animals had cysts in both the liver and lungs, only 6% of cattle had infection in the lungs alone. Hydatid-infected organs are routinely condemned and destroyed; this action represents considerable financial loss to abattoirs, since the arrangement in Australian abattoirs is offal becomes the property of the abattoir and the farmers are paid based on the weight of the carcass. The abattoirs where this study was conducted are now able to record multiple co-morbidities in slaughtered cattle, therefore, we were able to investigate the impact of hydatid infection, alone, on carcass weight. These data appeared to identify a minimal negative impact on carcass weight associated with infection, but this small difference (approximately 2.5kg) in infected animals was considered due to data-bias and not a real outcome of infection. Our data indicate hydatid infection in cattle does not appear to negatively impact weight gain. Nevertheless, our data show that hydatid disease in cattle is consistent with the distribution of dingoes along the Great Dividing Range and coastal areas where the climate is most conducive for long-term survival of *E. granulosus* eggs in the environment.



GENETIC DIVERSITY OF ECHINOCOCCUS GRANULOSUS SENSU STRICTO  
AMONG LIVESTOCK, WILDLIFE AND HUMANS IN FRANCE

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Cystic echinococcosis (CE) is a worldwide zoonosis caused by cestode species of the *Echinococcus granulosus sensu lato* (sl) complex. *E. granulosus sensu stricto* (ss) is the species responsible for the highest number of cases of CE in humans [1]. The present study aimed to increase our knowledge of the genetic diversity of *E. granulosus ss* in France in order to understand the circulation of the parasite in livestock, the interactions with wildlife, and potentially distinguish between autochthonous and imported cases of human CE.

129 sequences of the full *cox1* gene of the parasite were obtained from domestic animals (81 sheep, 19 cattle and one pig) collected at the slaughterhouse in France [2], from wildlife (10 wolf, one nutria and one chamois) and 16 human CE patients receiving surgery in France.

This study highlights a great genetic diversity and a low differentiation between sheep and cattle, as well as between geographical areas (North, Southeast and Southwest). A global predominance of the G3 genotype (67%) was described mainly represented by sheep samples from the main alpine historical focus. Among nine sheep farms in this region representing 51 samples, the number of haplotypes is generally increasing with the number of sheep infected with up to 6 haplotypes identified for 7 animals reflecting a high genetic diversity. Farms located less than 20 km from each other do not share common haplotype revealing that infection occurred separately in each farm but without excluding the potential role of transhumance. The common haplotypes found in livestock and wolves reflect the occasional prey predator relationship but remains insignificant in the maintenance of the lifecycle given the low occurrence of infection in wolves [3]. Concerning the cases of human CE linked to *E. granulosus ss*, the majority of the 16 cases analyzed would, as expected, probably as to be considered as imported cases with possibility to confirm the original region of infection (i.e. North Africa, Eastern Europe and Turkey). Nevertheless, as autochthonous human cases have already been reported, it is important to continue the genetic characterization of *E. granulosus ss* in both human and livestock to identify the potential epidemiological links and thus target action plans for the prevention and control of this parasite.

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# INVESTIGATION ON THE PREVALENCE OF HEPATIC CYSTIC HYDATID DISEASE IN BLACK-HEADED SHEEP IN BAYINBULAK AREA, HEJING COUNTY, XINJIANG IN 2021

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**Relevance.** To investigate the HCE (Hepatic cystic echinococcosis, HCE) infection of black-headed sheep in Bayinbulak area of Hejing County, Xinjiang by means of ultrasonic diagnostic screening.

**Materials and methods.** In July 2021, the current screening method was used in the Bayinbulak area of Hejing County, and the ultrasound and sonographic features of hepatic hydatidosis were used as the diagnostic criteria to investigate the prevalence of hepatic cystic hydatid in black-headed sheep. The HCE infection rates of sheep with different tooth ages were calculated, and the correlation between tooth age and infection rate was analyzed.

**Results.** The total infection rate of black-headed sheep grazing in Bayinbulak area of Hejing County was 31.6%. The infection rate of non-calcified HCE was 10.7%; the infection rate of calcified HCE was 20.1%. After grouping the black-headed sheep according to their tooth age, it was found that the HCE infection rate of the 1-2-year-old group was lower, and the HCE infection rate of the black-headed sheep  $\geq 3$  years of age was significantly increased, that is, the infection rate of HCE will increase with the increase of tooth age.

**Conclusion.** 1. The Bayinbulak area in Hejing County, Xinjiang is still a high-endemic area of HCE, and the infection rate of non-calcified CE is still high. HCE infection in black-headed sheep starts from the young age of sheep, and the incidence is high after 3 years of age. Young sheep should be the focus of preventing HCE in black-headed sheep. 2. In terms of methodology, convenient and practical inspection equipment and operating table, and flock preparation for finely prepared skins are all necessary conditions for improving screening efficiency and detection rate.

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# PILOT SURVEY OF CYSTIC ECHINOCOCCOSIS IN MASAAI LIVESTOCK-KEEPING COMMUNITIES OF NORTHERN TANZANIA

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**Relevance.** Prevalence mapping is among critical actions for cystic echinococcosis (CE) listed in the WHO 2021-2030 Roadmap for NTDs [1]. Knowledge of the epidemiological situation in sub-Saharan Africa is extremely scant. Maasai communities of northern Tanzania are suffering from a very high prevalence of cerebral coenurosis (*Taenia multiceps/Coenurus cerebralis*), causing high mortality in small ruminants [2]. Given the close similarity between the life cycle of *T. multiceps* and *E. granulosus*, this raises concerns about an increased risk of human CE. We aimed to estimate the prevalence of human abdominal CE and to investigate livestock infection in Maasai communities of Northern Tanzania, where no population-based ultrasound surveys have been carried out since the late 1980s [3].

**Materials and Methods.** Human CE was diagnosed by abdominal ultrasound in 5 communities of Longido and Ngorongoro districts. Infection in ruminants was evaluated through inspection in local abattoirs. 1-3 cysts/animal were collected and DNA extracted from 1 cyst/animal, prioritizing hepatic cysts. Molecular identification was performed using PCR followed by RFLP and Multiplex PCR. The COX1 PCR products of non-*E. granulosus s.l.* samples were sequenced [4].

**Results.** Ultrasound was performed on 823 volunteers; 6 hepatic CE cases, 3 of which with active cysts, were diagnosed in Ngorongoro (1.3% prevalence for this district). Of the 696 ruminants inspected, 34.2% had parasitic cysts. Molecular identification, achieved for 139 cysts, identified *Taenia hydatigena* in 48.2% and *E. granulosus s.l.* in 51.8%: *E. granulosus sensu stricto* (G1-G3) in 87.5% cases; *E. ortleppi* (G5) in 9.7%, and *E. canadensis* (G6-10) in 1 cyst.

**Conclusions.** Multiple *E. granulosus s.l.* species/genotypes are circulating in Maasai communities of northern Tanzania. Human CE was detected only in Ngorongoro, despite a high prevalence of cysts in livestock in both districts. More precise estimation of prevalence in this area and understanding of specific risk factors for CE among Maasai communities is needed, particularly given that cultural factors may be less of a barrier if some protective practices have already been adopted in other Maasai communities. Interventions targeting transmission routes common to both *E. granulosus* and *T. multiceps* would have dual benefits for preventing both human and livestock diseases. This study was funded by ESCMID Research Grant 2019. Molecular analysis was supported by funding from the EU Horizon 2020 Research and Innovation programme grant n.773830 (MEME project).

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## FROM CARNIVORE FECES TO HUMAN CASES: ENVIRONMENTAL AND FOODBORNE CONTAMINATION BY *ECHINOCOCCUS* EGGS

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The primary interest in *Echinococcus* generally focuses on its presence in definitive and intermediate hosts by detecting larval and adult stages of the parasite and obviously diagnostic and treatment in humans. The free stage, i.e. eggs, were concerned by far fewer studies regarding those targeting animals and humans despite the essential role in the lifecycle and as they constitute the only source of human infection. However, over the last decade, interest in environmental and food contamination by *Echinococcus*, as by other Taenidae species, has increased notably thanks to development of new methods.

The definitive hosts, mainly dogs and foxes, are the source of the environmental contamination for both alveolar and cystic echinococcosis. For several decades, taenid eggs have been characterized in studies by a potential long egg survival in the environment mainly impacted by temperature and humidity with a potentially high dispersal both of which contribute strongly to the maintenance of the lifecycles. An experimental and a spatio-temporal field study, both based on detection of *E. multilocularis* eggs in the soil will illustrate these characteristics. The high local contamination of soil by *Echinococcus* eggs is of particular interest regarding food contamination, especially as kitchen gardens were identified in France as hotspot for fox defecation, leading to a greater exposure of rodents and probably contamination of vegetables.

The human foodborne contamination by *E. multilocularis* and *E. granulosus* is both plausible and probable but has not yet been proved especially due to the long asymptomatic period. In order to provide data regarding food contamination, a multicenter study was realized among 1,034 lettuce samples, 300 strawberry batches and 130 blueberry batches from 13 European countries, but also Tunisia and Pakistan in the context of the EJPOH MEmE project. The detection of DNA of *E. multilocularis* and *E. granulosus sensu lato* in relevant proportions from different epidemiological situations represents a relevant step but is still not sufficient to confirm and estimate the contribution of food to human contamination. Further studies are now needed to obtain additional data on food but also on environmental matrices in different epidemiological situations worldwide notably with regard to confirmation of egg infectivity, in order to properly understand the sources of human contamination by *Echinococcus* eggs.

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## MULTICENTRE STUDIES FROM MEME PROJECT FOR THE DETECTION OF ECHINOCOCCUS SPP. IN DIFFERENT MATRICES

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Several multicenter studies have been conducted at European level and beyond in the MEmE project (One Health EJP consortium) [1]. These studies aimed to generate large-scale epidemiological data for the detection of *Echinococcus* spp. and to standardize molecular methods in different matrices. Three multicenter studies were conducted to detect *Echinococcus* spp. DNA in vegetables and dog feces and to compare different molecular methods in different matrices.

**Multicenter Study-1:** A multicenter study aimed to evaluate the proportion of lettuces and berries contaminated by *E. multilocularis* (Em) and *E. granulosus s.l.* (Eg) DNA. Specific real-time TaqMan qPCR assays were able to detect DNA from both parasites [2, 3] in pellets obtained after washing and filtrating [4] 1,034 lettuce samples, 300 strawberry batches and 130 blueberry batches from 13 European countries, but also Tunisia and Pakistan. DNA of Em and Eg was detected in a relevant proportion of samples. This finding is an indirect measure of environmental contamination by these zoonotic parasites. Whether these parasitic DNAs can be linked to the presence of infective eggs, and thus represent a real risk to humans, is currently unknown.

**Multicenter Study-2:** As dogs are considered a potential source of human infection, a multicenter evaluation of the prevalence of dogs infected with *Echinococcus* spp. and other Taenidae species was conducted on 1,619 faecal samples collected from seven countries. Parasite DNA was detected [2, 3, 5] in a small proportion of dogs (0.2% of Em, 1.4% of Eg and 4.0% of other Taenidae species). Questionnaires were also sent to dog owners to identify potential risk factors. Whether a relatively low prevalence in dogs may correspond to a high risk of infection for humans due to their close contact is currently unknown.

**Multicenter Study-3:** As molecular diagnostics are now essential for the detection of *Echinococcus* spp. DNA, a multicenter evaluation of three commonly used protocols [2, 3, 5] and three new techniques developed in MEmE was organized. Thirteen participants evaluated sensitivity and specificity by a blind testing of a panel of 20 copro DNA samples and 54 tissue DNA samples of *Echinococcus* spp. and other parasite species. The results were very homogeneous, indicating good robustness of the methods. Very satisfactory results were obtained on tissue samples with the two new methods based on conventional PCR combined with RFLP-based typing. Regarding the third new method [3], it confirms that real-time PCR assays are the methods of choice for copro-DNA.

**Acknowledgments.** This research was funded by the MEmE project from the EU's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme.

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## CONTAMINATION OF VEGETABLES FOR HUMAN CONSUMPTION BY ECHINOCOCCUS GRANULOSUS IN SARDINIA, ITALY (MeME project)

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**Background.** Promoting One Health in Europe through joint actions on foodborne zoonoses, in the framework of MeME European project, the contamination of about 1,200 vegetables, mainly lettuce, for human consumption by *Echinococcus multilocularis* and *Echinococcus granulosus sensu lato* (s.l.) has been evaluated in 10 European countries in summer 2021 (Umhang et al., 2022). The species *E. granulosus* s.l. belongs to genus *Echinococcus* and the family of Taeniidae. The larval stage of this tapeworm causes a zoonotic disease, the Cystic Echinococcosis (CE), a worldwide public health problem highly incident in all Mediterranean areas (Eckert et al., 2001).

The aim of this study was to detect the DNA of *Echinococcus* s.l., and/or other Taenidae species, presuming the eggs contamination of vegetables in non-intensive systems of a restricted but highly endemic area sited in the south of Europe, the Island of Sardinia (Italy).

**Material and Methods.** A total of 107 samples, mainly lettuces, were collected during the summer 2021, mostly from private producers and small local markets. An aliquot of 300gr (considering 50% limit of detection for 1 egg) of vegetables was washed within 3 days with 0.02% Tween 20 at WOA Reference Laboratory of Echinococcosis (Sardinia, Italy). While, successive filtering of the obtained pellets and molecular analysis by real-time PCR (Maksimov et al., 2020) and end-point PCR (Trachsel et al., 2007) were performed at Anses (Malzéville, France), along with sequencing analysis of the DNA isolates.

**Results.** A total of 4 lettuces were resulted contaminated by *E. granulosus sensu stricto* (s.s.) eggs. In addition, other Taenidae species were presented and identified as 3 *Hydatigera* spp., and 1 *Taenia multiceps*.

**Conclusion.** Our results have pointed to a potential source of infection related to the consumption of not properly washed raw vegetables. The zoonotic risk, higher in endemic areas, could be decreased or avoided by eating cooked or very well washed vegetables. However, further studies are needed to evaluate the viability of the eggs being still indeterminate.

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