

## Scientific Program

**Tuesday 5 July, 2005**

**09:00 - 09:20 Opening Ceremony**

Chairpersons: **Kiyoshi Kita** (Japan) and **Akira Ito** (Japan)

Sunao Yachiku (President, Asahikawa Medical College, Japan)  
Kiyoshi Kita (Professor, The University of Tokyo, President of Japanese Society of Parasitology, Japan)  
Mamoru Suzuki (President, Gunma University, Japan)  
Fujiro Sendo (President, Yamagata University, Japan)  
Maimati Yasen (President, Xinjiang Medical University, China)  
Akira Ito (Professor, Asahikawa Medical College, Japan)

**09:20 - 10:50 Japanese Contribution for Control of Parasitic Infections**

Chairpersons: **Antonio Montresor** (WHO/WPRO) and **Isao Tada** (Japan)

09:20 - 09:45 Global parasite control initiative of Japan (Hashimoto Initiative) (Somei Kojima, Japan) ..... **25**

09:45 - 10:10 Historical aspects for the control of soil-transmitted helminthiasis (Akio Kobayashi, Japan) ..... **26**

10:10 - 10:35 Strategy against parasitic infections of Japanese expatriates in developing countries (Atsuo Hamada, Japan)..... **27**

10:35 - 10:50 Comments on Japanese contribution for control of parasitic diseases (Antonio Montresor, WHO/WPRO) ..... **28**

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“All dressed up—but where are we going ? Progress and challenges in treatment and elimination of echinococcosis and cysticercosis”

Chairpersons: **Dominique A. Vuitton** (France) and **Ana Flisser** (Mexico)

**12:10 - 13:10 Lunch**

**13:10 - 15:00 Overview of Taeniid Cestode Zoonoses I. Taeniasis / Cysticercosis**

Chairpersons: **Hector H. Garcia** (Peru) and **Naoki Arizono** (Japan)

13:10 - 13:35 Present situation of taeniasis/cysticercosis worldwide (Antonio Montresor, WHO/WPRO) ..... **29**

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## Poster Presentation

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## **ALL DRESSED UP BUT WHERE ARE WE GOING ? PROGRESS AND CHALLENGES IN TREATMENT AND ELIMINATION OF ECHINOCOCCOSIS AND CYSTICERCOSIS**

Peter M. Schantz

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Larval cestode diseases, particularly the echinococcoses, cystic (CE) and alveolar (AE), caused by the larval stages of *Echinococcus granulosus* and *E. multilocularis*, respectively, and cysticercosis caused by larvae of the pork tapeworm, *Taenia solium*, respectively, are well known by the medical community because of their often dramatic clinical presentation and complexities of diagnosis and clinical management. Surgical interventions and extended chemotherapy are current methods of treatment of these infections that remain prevalent throughout vast areas of the world. Where adequate care is available the treatment of individual cases can cost thousands of dollars. The impact of these diseases on patients/persons diagnosed with the disease is appreciated by the medical community but the broader impact of these infections on individuals and the affected communities has been largely unknown or ignored. These infections have impact on the health and productivity of both humans and lower animals. Assessing the economic impact is especially difficult because it comprises human and animal health as well as agriculture, trade and market factors. These costs associated with diagnosis and medical care are combined with the concomitant animal health losses to amplify the total economic losses to society. Control and elimination of these diseases will result in considerable benefits to affected populations. The past two decades have been marked by remarkable progress in science and technology which has provided marvelous insights into the biology, epidemiology and medical aspects of diseases caused by larval cestodes. We have seen the development of organ-scanning technology which permits the noninvasive examination and diagnosis of cestode larval lesions in any site of localization as well as sensitive and specific immunologic technology which permit screening and confirmation of diagnosis in remote populations. We now are able to detect specific tapeworm infections in canine and human definitive hosts by detection of specific antigens in stools and serum antibodies, distinguishing adult- and larval-stage infections. Application of this new diagnostic technology has provided stunning information concerning the distribution, prevalence and burden of these infections. Innovative technology is now widely available to participants in the international collaborative research networks and, in many cases, is available more widely for clinical and epidemiological applications through commercial sources. Current information and technology refocuses and expands our concerns from diagnosis and medical care of these infections when imported into advanced countries in immigrants from endemic areas to applications in efforts to better understand the biology and epidemiology of these infections in the endemic/epizootic settings. Much of this progress has been achieved through international collaborations fostered by participants in this international conference.

## ROLE OF CHEMOTHERAPY OF TAENIASIS IN PREVENTION OF NEUROCYSTICERCOSIS

Zbigniew Pawlowski

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Human carriers of the adult tapeworm, *Taenia solium*, are the sole sources of cysticercosis for other humans and pigs. Therefore, a key intervention in preventing neurocysticercosis is elimination of taeniasis by chemotherapy. Other control measures such as sanitation, ban on using human faeces as a fertilizer or food for pigs, vaccination of pigs, chemotherapy of pigs, meat inspection, proper handling of pork and health education are of an auxiliary importance. Implementation of these non-chemotherapeutic measures led to a near eradication of taeniasis/cysticercosis in Central Europe in the early XX century, before safe and effective taeniocides were available. Currently, however, we have two modern non-toxic taeniocides, namely niclosamide and praziquantel, with taeniocidal efficacy of 85% and 95%, respectively. Both drugs are on the WHO Essential Drugs list. Treatment with praziquantel is inexpensive (10 US cents/dose). Theoretically, intervention using these taeniocides could make the control of *T. solium* taeniasis/cysticercosis both simple and feasible, however, there remain several practical obstacles to successful intervention. These include diagnosing *Taenia* carriers, availability of drugs and prioritization of neurocysticercosis for control. Nevertheless (1) problem with diagnosis of taeniasis can be overcome by effective health education and wider use of newly developed coproantigen tests; (2) lack of availability of modern taeniocides, where needed, can be remedied by strengthening health services infrastructure; (3) priority given to prevention neurocysticercosis amongst health policy makers can be upgraded by emphasizing its public health importance, mainly, as a leading cause of late epilepsy. Information is accumulating on rational population based control measures. The short-term aim of control would be to lower the mortality, morbidity and disability due to taeniasis/ cysticercosis since effective eradication in most endemic regions remains a goal for the distant future. The areas, where cysticercosis is prevalent are known or can be identified. In these areas chemotherapeutic intervention should cover all, confirmed or suspected cases of *Taenia* spp. taeniasis. Any treated case of taeniasis decreases the *T. solium* pool and consequently lowers the local incidence rate of neurocysticercosis. Focus-oriented chemotherapy is more rational than mass-treatment. The definition of a focus has been elaborated. Vertical approaches are not essential, as horizontally organized control measures, using existing medical, veterinary and public health services, may work effectively, if implemented and supported by communities.

## GLOBAL PARASITE CONTROL INITIATIVE OF JAPAN (HASHIMOTO INITIATIVE)

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Parasitic diseases, including cysticercosis and echinococcosis, still remain to be major health hazards to health of people, especially those living in tropical developing countries. Once called as the paradise of parasites just after the Second World War, Japan succeeded to eliminate or eradicate major parasitic diseases such as malaria, filariasis, and schistosomiasis in addition to soil-transmitted helminthiases (STH) in 30 years thereafter. It should be noted that this successful story was initiated by using school health-based approach as an entry point to the community, and that this approach was carried out through triangular cooperation among governmental and community-based non-governmental organizations, and scientific expert communities. Based on this Japan's experience, in 1997 and 1998, on the occasion of the G8 countries' summit at Denver and Birmingham, respectively, then-Prime Minister of Japan, H. E. Dr. Ryutaro Hashimoto, pointed out the importance of parasitic diseases control in improvement of public health and proposed the necessity of strengthening international cooperation toward global parasite control. Since lack of human resources is one of the major obstacles to overcome these health hazards in most of developing countries, the Government of Japan announced her policy at the Tokyo International Conference on African Development (TICADII) in 1998, which proposed to establish three centers for research and training, one in Asia and two in Africa. This action was further strengthened by the Okinawa Infectious Diseases Initiative in 2000. Thus, the Asian Centre of International Parasite Control (ACIPAC) was established in Bangkok in March 2000 as a JICA technical cooperation project in collaboration with the Mahidol University and the Ministry of Public Health, Thailand. The present paper describes ACIPAC's activities of the past five years, which were carried out being focused on the establishment of the concept of school health-based parasite control in the Great Mekong sub-region countries. In addition to contributing human resource development through its training courses on school-based malaria and STH control for program managers as well as small-scale pilot projects implemented in partner countries, ACIPAC proposed also that schoolchildren should be considered as health partners rather than simple recipients of health services including deworming programs.

# HISTORICAL ASPECTS FOR THE CONTROL OF SOIL-TRANSMITTED HELMINTHIASES

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<sup>1</sup>Jikei University School of Medicine, Tokyo, Japan and <sup>2</sup>Japan Association of Parasite Control, Tokyo, Japan

Japan is one of the very few countries that operated the nation-wide control programme against soil-transmitted helminths (STH) infections and gained a great success. The Japan's knowledge and experience about the control strategy were transferred to Asian countries as integrated project (IP) through APCO research group and also to countries of Asia, Africa and Latin-America through the seminar and workshop on parasite control administration.

## **1. The nation-wide parasite control programme in Japan**

After the World War II, STH infections occurred explosively among Japanese people, peaked at 73% in 1949. Under a good tripartite cooperation among government, experts and private sector organization "selective mass-treatment targeted at school children" was adopted as the "main control strategy". Epidemiological studies suggested that mass-examination and selective mass-treatment should be made twice a year. A newly invented mass-examination method, cellophane thick smear (Kato technique) was applied by Japan Association of Parasite Control (JAPC) in the nation-wide STH control programme. Fee-charging services expanded and sustained the activities of JAPC and its 37 branch associations on a self-supporting basis. Thus, prevalence rates of STH infections among the people have decreased to the eradication stage by 1970.

## **2. Asian Parasite Control Organization (APCO)**

APCO was inaugurated in 1974 by the late Mr. Kunii in an idea of integrating STH control with family planning and nutritional improvement (IP) in which STH control was expected as a catalyzer. The APCO parasitologists' group was composed of 13 countries; Japan, Korea, Taiwan, Indonesia, Philippines, Thailand, Malaysia, Sri-Lanka, Nepal, Bangladesh, China, Viet-Nam and Laos. Their studies covered various areas in relation to parasite control, bearing IP in mind. The regional training course has been held every year in Mahidol University, Thailand. The yields of their studies and achievements are extremely valuable and were published in a series of "Collected Papers on the Control of Soil-transmitted Helminthiases", Vol. I to VII.

## **3. Seminar on Parasite Control administration for Senior Officers**

### **- A step toward primary health care -**

This seminar has been held every year since 1978, where lectures and field observation tour are designed. The seminar coupled with APCO meeting have greatly contributed to those countries in their IP and parasite control projects, and furthermore in setting-up of Workshop on the Global Parasite Control Administration, Hashimoto Initiative, in 1999.

## **STRATEGY AGAINST PARASITIC INFECTIONS OF JAPANESE EXPATRIATES IN DEVELOPING COUNTRIES**

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According to statistics from the Ministry of Foreign Affairs in 2002, about 200,000 Japanese people live in developing countries, and are at risk of various parasitic infections. In order to lower the risk of the infections, we conducted two surveys.

### **Survey of intestinal parasitic disease**

To clarify the prevalence of intestinal parasitic infections among Japanese expatriates of developing countries, we examined their fecal specimen annually from 1995 to 2000 by formalin-ether sedimentation method. In 1995, the infection ratio was 3.0% (n=981), decreasing to 1.6% (n=1,574) in 1998. However, the ratio began to increase in 1999 (2.0%, n=1,713) and 2000 (2.5%, n=,806). The ratio in Africa was the highest each year, followed by Asia and Latin America. Heterophyidae (51 cases), *Giardia lamblia* (42) and *Trichuris trichiura* (30) were detected most frequently. In Egypt, the ratio of Heterophyidae rose by 28.8% in 2000. This was the prime reason for the increase in the overall ratio of infections worldwide.

### **Survey of malaria**

To clarify the current usage of chemoprophylaxis against malaria for Japanese expatriates, we distributed questionnaires to Japanese living in tropical Africa annually from 1998 to 2002. Analysis showed that the use of chemoprophylaxis was 23.1% in 1998 (n=91), but the percentage decreased to 13.8% in 1999 (n=80). From there, the percentage dropped dramatically to 2.2% in 2000 (n=136), 1.6% in 2001 (n=127) and 4.5% in 2002 (N=66). To find out the reason for this abrupt change, we issued another questionnaire in 2002. Out of 66 who responded, 17(25.8%) had suffered from malaria within the last year. Although more than 50% used some sort of preventive measures, the majority used milder measures such as protection against mosquito bites and stand-by treatment because of concerns about side effects of chemoprophylaxis.

### **Conclusion**

Even today parasitic infections are a major health hazard among Japanese expatriates in developing countries. In order to eradicate the infections from this group, we must continue preventive measures such as health education.

## COMMENTS ON JAPANESE CONTRIBUTION FOR CONTROL OF PARASITIC DISEASES

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The Japanese contribution to soil-transmitted helminth (STH) control has been of enormous importance since demonstrated the feasibility of chemotherapy-based control programmes. The Japan Association of Parasite Control is one of the best model of the proper utilization of the available resources and creative development of appropriate methods. The health school law (1958) is still now one of the best examples of political commitment to public health. The results obtained (*Ascaris lumbricoides* prevalence drop from 63% in 1949 to 0.9% in 1990) are even more outstanding because obtained with drugs that were not completely free of side effects, not single dose and were much more expensive than the ones presently available.

WHO considers that three major improvements occurred in the last 15 years in this field that have further facilitate chemotherapy-based control programmes for STH:

- The development of single dose/single administration anthelmintic
- The development of drugs that is not absorbed and therefore extremely safe and administrable by not medical personnel.
- The progressive reduction of cost (presently mebendazole 500 mg is available, in generic formulation, at less than 0.02 USD/dose)

To enable countries with extremely limited resources to implement chemotherapy-based control programmes for STH, WHO developed a simple strategy that consist in three steps:

- Rapid epidemiological evaluation (allowing to classify the areas of the country in different categories according to the prevalence of STH)
- Administration of deworming to the schoolchildren in high endemic areas by non medically trained personnel
- Support of the deworming activities with health education and where possible improvement of sanitation standards

Following this simple strategy it is possible to reach a very large number of schoolchildren with very limited funds. For example in 2004 the MoH of Cambodia covered 96% of all the school-age children of the country (2,770,000 children) at cost of 160,000 USD (treated child cost 0.033 USD). The costs were covered by the MoH budget and WHO provided technical assistance. This experience demonstrates that, where political commitment is present, the WHO strategy is achievable even in countries like Cambodia that ranks 130th out of 175 on the human development index. Where additional human and financial resources are available more elaborated strategies are also encouraged by WHO.



## **PRESENT SITUATION OF TAENIASIS/CYSTICERCOSIS WORLDWIDE**

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Pig production has increased significantly worldwide especially in rural and resource-poor communities. Recent reports from Africa and Asia document that concurrently with the increase of this pig keeping and pork consumption there have been increase of porcine cysticercosis. In particular East Africa countries report among the highest prevalence in the world (up to 56% prevalence in Zambia). A parallel increase of human neurocysticercosis is expected but recent data are not available from most of African countries and are very limited and sparse data are available for Asian countries. Most of the information on neurocysticercosis and control experiences came from Latin America: where research on the disease has gained interest and the recommendations developed obtained political support. Studies conducted in that continent show that control measures should be focused on tape-worm carries: however large scale parasitological screenings are not feasible in most endemic countries for economical reasons and large scale mass treatment is not feasible for the risk of generating neurological symptoms in person with previous occult neuro-cysticercosis.

The strategy that is at the moment more promising is a combination of the different tools available and includes:

- (1) Identification of area at risk by surveillance of epilepsy cases
- (2) Questionnaire-screening of the population in the areas at risk
- (3) Presumptive treatment under supervision of the suspected cases and their families.

This active finding and treatment of probable tape-worm carries should be accompanied by health education (prevention and self diagnosis) and control swine cysticercosis (vaccination or routine treatment with 1 day praziquantel before slaughtering).

This strategy is extremely cost-effective because prevent a large number of cysticercosis epilepsy that is a debilitating disease and require extremely expensive treatment once established.

WHO invite all endemic countries to recognize the importance of teniasis/cysticercosis control and to collect epidemiological data and to adopt policies and strategies for its rationale control.

## **TAENIA SOLIUM CYSTICERCOSIS IN THE INDIAN SUBCONTINENT: PROBLEMS, PRIORITIES AND PROSPECTS FOR FUTURE STUDY**

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The parasitic infestation of the human brain by the pork tapeworm, *Taenia solium*, known as neurocysticercosis (NC) is well-recognized by clinicians and lay people alike in the Indian subcontinent. Regrettably, virtually all epidemiological information available about the disorder arises from hospital-based case series and anecdotal reports. These reports do not represent the true proportions of infection; nevertheless they indicate that within the subcontinent, NC remains common in most of India apart from the northern state of Kashmir and the southern state of Kerala. It is also commonly reported from Nepal; however there are no reports from Pakistan, Bangladesh and Sri Lanka. In an analysis of a large sample of seizure disorders (n=1,026), comprising of single seizures, newly diagnosed epilepsy, prevalent epilepsy and acute symptomatic seizures presenting to a secondary-tertiary care hospital in Northwest India, NC was diagnosed in 34.6%. Evidence of NC was noted in 59.2% of those with a single seizure, 92.0% of those with recurrent acute symptomatic seizures, none of cases of incident epilepsy and 2.0% of prevalent epilepsy. A diagnosis of NC was significantly associated with single seizures ( $p<0.001$ ). Certain clinical features such as the occurrence of seizures in clusters, focal symptoms and post-ictal neurological deficits could be used in order to develop of discriminant model for predicting a diagnosis of NC among people presenting with seizures to the hospital. The diagnostic utility of serology, when examined in a nested sub-sample as well in other published reports was found to be modest. It is desirable to examine the predictive value of discriminating clinical features of seizures due to NC in conjunction with serology in a prospective community-based study. Thus, whilst, hospital-based studies such as the one presented herein are useful in improving our understanding of the disorder, field studies examining the burden of seizure disorders due to NC and its ecological determinants are much required.

## **PRESENT SITUATION OF TAENIASIS/CYSTICERCOSIS IN THE AMERICAS**

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*Taenia solium* is endemic in most countries of Central and South America, and is increasingly diagnosed in the USA because of globalization and increased migration. Reports on prevalence of taeniasis and cysticercosis are now available for most countries, and recently, several articles from different countries in South and Central America consistently showed an association between neurocysticercosis (NCC) and seizures and or epilepsy, with a gross estimate of attributable fraction of around 30% of all seizures. An interesting feature is the frequency of viable brain cysts in opposition to the profile of clinically manifest cysticercosis in India. Cysticercosis is potentially eradicable, and control interventions are underway to attempt to eliminate this infection. Several attempts to control transmission in the field have been performed in Ecuador, Mexico, Guatemala, and Peru, among other countries. A wide field based program to demonstrate the feasibility of eliminating cysticercosis is underway in a province of Peru, funded by the Bill and Melinda Gates Foundation. While sustainability can be foreseen as the major obstacle, other problems include the lack of basic sanitary facilities in endemic areas, the extent of domestic pig raising (based on free-ranging animals and thus no food investment), the costs of the interventions, and most importantly, their cultural acceptability.

## PRESENT SITUATION OF TAENIASIS/CYSTICERCOSIS IN AFRICA

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Mounting evidence indicates that *Taenia solium* infections are widespread throughout sub-Saharan Africa in areas where pig keeping is practised and pork consumption occurs. More than 20 countries are considered endemic. Coincident with the emerging problem of cysticercosis in the region is increasing popularity of pig keeping in rural and periurban smallholder communities and pork consumption in both rural and urban areas as well as high and increasing prevalences of epilepsy. Investigations on the presence and impact of cysticercosis in the region have been hindered by a serious lack of financial resources and technical capacity, resulting in the absence of an evidence base from which decision makers could set appropriate priority for combating cysticercosis. In those countries where epidemiological studies have been undertaken results indicate important similarities and differences within as well as outside the region with regard to transmission and risk factors. Important findings from the Africa region include congenital infection of porcine cysticercosis, canine cysticercosis as a possible source of infection in dog meat-consuming areas of western Africa and a high prevalence of juvenile neurocysticercosis in South Africa. A recent global assessment of the burden of cysticercosis commissioned by WHO estimated that the economic impact of *T. solium* infections in just one province of South Africa (Eastern Cape Province) to be more than US \$15 million when both the health and agricultural costs were considered comprehensively. Losses due to porcine cysticercosis alone in western and central Africa have been estimated to be more than 25 million euros. In 2000 a group of cysticercosis researchers from Eastern and Southern Africa formed a regional working group (CWGESA) aimed at promoting integrated research and control efforts for cysticercosis. The CWGESA held an international workshop in 2002 on the emerging cysticercosis situation in eastern and southern Africa which culminated in the formulation of a regional action plan for combating cysticercosis. Current attention is being directed at increasing awareness among various stakeholder groups and establishing regional reference and training centers in order to strengthen local capacity for understanding and handling the cysticercosis problem in Africa.

## PRESENT SITUATION OF ECHINOCOCCOSIS IN EUROPE

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*Echinococcus multilocularis*, causing alveolar echinococcosis (AE), is recently observed to intensify its transmission in Europe. This is apparent from increased prevalence rates in foxes (*Vulpes vulpes*) in Central Europe, new foci of high endemicity (e.g. in Poland and Slovakia), and newly recognized endemic areas (e.g. northern Italy, Hungary, and western Belgium). In addition, the transmission cycle is increasingly getting established in urban areas, where the population density of foxes can be >5 times higher than in rural landscapes. The role of neozootic mammals as additional hosts needs to be monitored. Especially raccoon dogs (*Nyctereutes procyonoides*) are rapidly increasing their populations in Poland and eastern Germany, and may in future serve as an additional pool of definitive hosts. Based on recent surveys, *E. multilocularis* still seems to be absent in Britain and Fennoscandia. However, accidental introductions can occur rapidly, as demonstrated by the example of Svalbard where the lifecycle got established after the introduction of a neozootic vole species. Cystic echinococcosis (CE) is by now known to be caused by a cluster of *Echinococcus* species. *E. granulosus* s.str. (G1) still occurs at high prevalence rates in parts of the Mediterranean region (e.g. Spain, Sardinia), where its presence in animals correlates with considerable incidences of human CE. A re-emergence of *E. granulosus* was recently reported from Wales after the large scale outdoor slaughter of sheep following an outbreak of foot and mouth disease in 2001. Other taxa of the *E. granulosus* cluster (*E. equinus*, *E. ortleppi*, *Echinococcus* spp.) have received less attention, since they appear to a lesser degree to be associated with human disease. In Finland, the endemic, cervid strain' (G10) was shown to be genetically distinct from the North American form (G8). Wolves appear to be increasingly involved in transmission of this taxon.

## ***ECHINOCOCCUS GRANULOSUS* IN AUSTRALIA, WIDESPREAD AND DOING WELL !**

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Since its arrival into Australia with European settlers about 200 years ago *Echinococcus granulosus* has become widely established in wildlife through a predator/prey interaction mainly between dingoes (*Canis lupus dingo*) and various species of macropodid marsupials. However, several other species (feral pigs (*Sus scrofa*), foxes (*Vulpes vulpes*) and wombats (*Vombatus ursinus*)) may also be involved. Although common and shown to be susceptible to infection experimentally, introduced European rabbits (*Oryctolagus cuniculus*) are not part of the transmission pattern of *E. granulosus* in Australian wildlife. Preliminary data also suggest members of the Family Dasuridae, native marsupial carnivores, (namely the eastern quoll, (*Dasyurus maculates*)) seem refractory to infection with *E. granulosus*. The largest member of the Dasuridae, the thylacine or Tasmanian 'tiger' (*Thylacinus cynocephalus*) is extinct on the Australian mainland, having been out competed by dingoes since their arrival from Asia with Asian seafarers 4,000-5,000 years ago. Had dingoes never reached Australia and the thylacine been present as the wildlife top-order predator at the time of European settlement, it is debatable if *E. granulosus* would have ever established in wildlife. More recently, changes in behaviour of dingoes in foxes in some areas of Australia have *E. granulosus*-infected dingoes and foxes entering urban centers. Currently in Australia, wildlife is an important reservoir of infection providing a constant source of hydatid infection to domestic livestock and humans and a source of echinococcosis for domestic dogs. The implications of wildlife reservoirs for effective control of *E. granulosus* will be discussed.

## ECHINOCOCCOSIS IN NORTH AMERICA

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Both cystic and alveolar forms of echinococcosis occur in the Americas. *Echinococcus multilocularis*, the agent of alveolar echinococcosis is limited to North America. Formerly it was a chronic public health threat in northern communities of Native Americans in Alaska and human cases were diagnosed regularly until the late 1980s. An intervention based on public health educations, periodic anthelmintic treatment of dogs and improved housing was implemented in the mid 1980s and no new infections have been diagnosed in Alaska since 1989. A large and possibly expanding focus of *E. multilocularis* has been noted in the Northcentral U.S.A. involving foxes and coyotes and their rodent prey. Infection rates in sylvatic hosts is high, however, there has been only a single human case diagnosed in association with that focus. Nevertheless, the situation remains of public health concern because of the possibility for further spread and potential exposure of humans.

Cystic echinococcosis (*E. granulosus*) infection has occurred widely in the United States and Canada. Clinical cases have always, and continue to be, primarily imported in immigrants from countries where the infection is endemic. Endemic foci of transmission exist in several regions, however. In the north (Alaska and Canada) the sylvatic form of *E. granulosus* occurs in wolves and cervids. Humans become exposed primarily from dogs fed the viscera of infected cervids killed during hunting. Foci of transmission of *E. granulosus* in domestic animal hosts have been described in several regions of the United States. In California transmission in the sheep dog cycle was noted in the Central Valley of California, among sheep herders in Utah, and among Native American populations in Arizona and New Mexico. Recent investigation suggest that transmission no longer occurs in California and Utah, however, cases continue to be diagnosed regularly in Arizona and New Mexico suggesting continued active transmission.

## PRESENT SITUATION OF CYSTIC ECHINOCOCOSIS IN SUB-SAHARAN AFRICA

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Sub-Saharan African is situated 20<sup>0</sup> north and 35<sup>0</sup> south of Equator. It has an area of approximately eight million square kilometers and an approximately population of about 750 million people. The biomes of this region comprise mainly of deserts (Sahara, Kalahari and the Horn of Africa); tropical rain forests located in western and central Africa, and tropical savannah. The Sahel, eastern Africa and most of the southern Africa are dominated by savannah grasslands. The Savannah is home to the most diverse group of large mammals remaining on the earth. The African Savannah is characterized by the picturesque baobab and thorny acacia trees. This savannah support herds of many species of antelopes, wildebeests, elephants, zebras, giraffes, lions and cheetahs. It is one of the last places where one may witness a world that our prehistoric ancestors might have known.

Cystic echinococcosis (CE) is highly endemic among the nomadic pastoral tribes of East Africa, but is rare amongst the agriculturally based communities. Human CE have been reported among Maasai community of southern Kenya and adjacent northern Tanzania, and, most notably among the pastoral tribes of northwestern Kenya (Turkana), north eastern Uganda (Karamajong), southern Sudan (Toposa, Boya, Lotuka) and southern Ethiopia (Nyangatom, Merille).

In Turkana District, the estimated prevalence in the population approaches 7%, a rate which is only found elsewhere in parts of South America and China. *Echinococcus granulosus* infections are common in dogs from all countries where they have been examined. Transmission of *E. granulosus* to humans is affected by such factors as prevalence of the parasite in domestic dogs, behaviors of humans towards dogs, and heterogenicity of the parasite and susceptibility of humans to infection. Sheep and goats appear to be the most common domestic intermediate hosts, but recent studies suggests that camels are equally important intermediate host, especially in Sudan. At least seven of nine *E. granulosus* genotypes are infective to humans in sub-Saharan African. Most human cases of CE are caused by the sheep strain (G1) and camel strain (G6) of *E. granulosus*. Other strains occurring in the area are the lion strain, horse strain and cattle strain (G5, *Echinococcus ortleppi*).



## **PRESENT SITUATION OF ECHINOCOCCOSIS IN THE MIDDLE EAST AND ARABIC NORTH AFRICA**

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Echinococcosis is one of the major zoonotic parasitic diseases in the Middle East and Arabic North Africa from Morocco to Egypt. Both cystic and alveolar echinococcosis has been reported from these areas. However, cystic echinococcosis is more prevalent and has been reported from all countries in the Middle East and Arabic North Africa. Alveolar echinococcosis is less prevalent and has been reported only from Iran, Turkey, Iraq and Tunisia. Present situation of echinococcosis in dogs and other definitive hosts, animal intermediate hosts and humans in the Middle East and Arabic North Africa has been reviewed. The prevalence of *Echinococcus granulosus* is high in Iran, Turkey, Iraq, Morocco, Tunisia, and Libya. In Jordan, Palestinian Autonomy, Israel, Syria, and Lebanon, the cystic echinococcosis is also highly endemic. In Oman, it is endemic, although the prevalence is low and in Cyprus very low level. Various surveys have indicated that hydatid cysts are commonly found in sheep, cattle, goats and camels throughout the countries in Middle East and Arabic North Africa. In many countries sheep are the most infected animal. In human, most of studies have been focused on surgical reports although several population studies have been made using serological and imaging techniques. Human cystic echinococcosis (CE) is prevalent in Middle East and Arabic North Africa. It is hyperendemic in Iran, Turkey, Iraq, Jordan, Morocco, Libya, Tunisia, and Algeria, and endemic in Egypt. Studies on the strain specificities of *E. granulosus* in the Middle East revealed two strains of *E. granulosus*: the sheep strain (G1) in sheep, goats and human patients, and the camel strain (G6) in camels as well as human. Dog/sheep strain is to be more prevalent. Dog/camel strain has also been identified in human cases. G1 strain (sheep/dog) is prevalent strain in Jordan. However, *E. granulosus* resembling the G4 strain has been reported from Jordan. Strain specification of *E. granulosus* in Arabic North Africa has showed that sheep/dog strain (G1) have been reported from Tunisia and Libya both from human and animals. However, the human cases in Egypt are the camel/dog strain.

## PRESENT SITUATION OF CYSTIC ECHINOCOCCOSIS IN CENTRAL ASIA

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Cystic echinococcosis (CE) caused by *Echinococcus granulosus* has always been an endemic disease in the former republics of Central Asia. During the period of Soviet Administration up to 1991, human surgical incidence rates tended to be relatively low with perhaps at most 1-5 cases per 100,000 per year. Following the collapse of the Soviet Union and the emergence of the New Independent States there has been profound economic and social changes. Associated with this has been a serious epidemic of CE throughout the region. In many areas figures suggest the surgical incidence is now greater than 10 cases per 100,000. Furthermore, official government figures are believed to substantially under report the extent of the problem. For example, official figures in Uzbekistan reported 819 cases of CE surgically treated in 2001. However, a detailed analysis of hospital records suggests that the true figure was 4089, more than four fold higher. The latter figure represents an annual surgical incidence rate of nearly 25 cases/100,000 per year. Similarly high endemic areas are seen in southern Kazakhstan, Kyrgystan and Tajikistan with incidence rates of up to 13 cases/100,000, 20 cases /100,000 and 27 cases/100,000 respectively. A disproportionate number of cases are in children and the unemployed. The rates of infection have also increased in major livestock species such as sheep with a doubling of reported prevalence in some areas. In the dog population, independent studies in Uzbekistan and Kazakhstan have demonstrated that the rural dog population, closely associated with the sheep industry are highly infected, with prevalence rates approaching 25%. Village and urban dogs have considerably lower prevalence rates.

## DISTRIBUTION AND ECOLOGY OF *ECHINOCOCCUS MULTILOCULARIS* IN CENTRAL ASIA

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Natural endemic foci of *Echinococcus multilocularis* in Central Asia occur in the southern part of an area of the parasite, where the dry steppe and desert prevails. Therefore in extensive areas there are no favorable conditions for the survival of cestode eggs in the environment. There are large numbers of intermediate and definitive hosts throughout the area and in defined localities in the steppe and desert and in many mountainous regions a high prevalence of infection is found in both predators and rodents.

In deserts foci of *E. multilocularis* are very patchy and are associated with colonies of gerbils (*Rhombomys opimus*). The populations of these rodents are high throughout the desert. High infection rates of *E. multilocularis* in gerbils are found in the river Emba valley (3.1 %) and the Ural River valley (1 %) in West Kazakhstan. To the northwest of the Aral Sea and in the Ustiurt plateau the infection rates are 0.21-0.31 %. Here foci of infection are seen in rodent colonies associated with *tape*, *oasis* and *island* habitats where there are favorable soil conditions. In particular moisture is present in the spring. In the deserts of Kzylkum, Moinkum and Southern Balkhash this parasite has not been found.

In steppe 8-10 species of rodent participate in the formation of endemic foci of alveolar echinococcosis with infection rates of 0.4-4.6 %. Infection rates of red foxes (*Vulpes vulpes*) in the steppe have been recorded at 25.9 % and 21.7 % in the corsak fox (*Vulpes corsak*). In the steppe there are four types of endemic foci, characterized by their location in respect to host habitat. It appears that *small forest*, *lakeshore*, *valley-meadow* and *low-lying steppe* habitats result in constant circulation of the parasite.

In mountains high infection rates of alveolar echinococcosis are found in subalpine meadows, summer pastures, the banks of streams and small rivers, lakeshores and foothill plateaus. Infection rates of rodents (voles, marmots) are 0.7-8 % and 17-22 % in red foxes.

In formation of endemic foci of alveolar echinococcus landscape determinants are important. These include soil structure, presence or absence of moisture, depth of vegetation cover and type of vegetation. These determine the survival rate of eggs and the interactions of intermediate and final hosts.

## MOLECULAR SURVIVAL STRATEGY OF *ECHINOCOCCUS MULTILOCULARIS* IN THE MURINE HOST

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Larval infection with *Echinococcus multilocularis* starts with the intrahepatic postoncospherical development of a metacestode that – at its mature stage - consists of an inner germinal and an outer laminated layer (GL & LL). In certain cases, an appropriate host immune response may inhibit parasite proliferation. Several lines of evidence obtained by *in vivo* and *in vitro* experiments indicated the important bio-protective role of the LL. Among others, the LL was proposed to protect the GL from nitric oxide produced by peri-parasitic macrophages and dendritic cells, and also to prevent immune recognition by surrounding T cells. On the other hand, the high periparasitic NO production by peritoneal exsudate cells increases periparasitic immunosuppression, explaining why iNOS deficiency confers a certain degree of resistance against infection in mice. The intense periparasitic granulomatous infiltration indicates a strong host-parasite interaction, and the involvement of cellular immunity in controlling the metacestode growth kinetics is strongly suggested by experiments carried out in T cell deficient mouse strains. Carbohydrate components of the LL, such as Em2 (G11) and Em492, as well as parasite metabolites yield immunomodulatory effects that allow the parasite to survive in the host. I.e., the IgG response to the Em2 (G11)-antigen takes place independently of  $\alpha\beta^+CD4^+$  T cells, and in the absence of interactions between CD40 and CD40 ligand. Such parasite molecules also interfere with antigen presentation and cell activation, leading to a mixed Th1/Th2-type response at the later stage of infection. Furthermore, Em492 and purified parasite metabolites suppress ConA and antigen-stimulated splenocyte proliferation. Infected mouse macrophages (AE-MØ) as antigen presenting cells (APC) exhibited a reduced ability to present a conventional antigen (chicken ovalbumin, C-Ova) to specific responder lymph node T cells when compared to normal MØ. As AE-MØ fully maintain their capacity to appropriately process antigens, a failure in T cell receptor occupancy by antigen-Ia complex or/and altered co-stimulatory signals can be excluded. Studying the status of accessory molecules implicated in T cell stimulation by MØ, it could be shown that B7-1 (CD80) and B7-2 (CD86) remained unchanged, whereas CD40 was down-regulated and CD54 (=ICAM-1) slightly up-regulated. FACS analysis of peritoneal cells revealed a decrease in the percentage of  $CD4^+$  and  $CD8^+$  T cells in AE-infected mice. Taken together the obstructed presenting-activity of AE-MØ appeared to trigger an unresponsiveness of T cells leading to the suppression of their clonal expansion during the chronic phase of AE infection.

## **SURVIVAL STRATEGY OF *ECHINOCOCCUS MULTILOCULARIS* IN THE HUMAN HOST**

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As exemplified by the “aborted” calcified liver lesions found in endemic areas, only a minority of individuals among humans does allow the development of *Echinococcus multilocularis* metacestode. Clinical research has disclosed some aspects of the survival strategy of *E. multilocularis* in human hosts. Clinical observations in liver transplantation and AIDS suggest that suppression of cellular / Th1-related immunity increases disease severity. Most of the studies have stressed a role for CD8<sup>+</sup> T cells and for Interleukin-10 in the development of a tolerance state. A spontaneous secretion of IL-10 by the PBMC seems to be the immunological hallmark of patients with progressive forms of alveolar echinococcosis (AE). IL-10-induced inhibition of effector macrophages but also of antigen-presenting dendritic cells may be operating to protect the parasitic growth and survival. The genetic correlates of susceptibility to *E. multilocularis* are clearer in humans than in the mouse model. A significant link between MHC polymorphism and clinical presentation of AE has been shown, and the spontaneous secretion of IL-10 in patients with a progressive AE is higher in patient with the HLA DR3<sup>+</sup>, DQ2<sup>+</sup> haplotype. Clustering of cases in certain families, in communities otherwise exposed to similar risk factors, also points to immuno-genetic predisposition factors that may allow the larva to escape host immunity more easily. The first stage of larval development might be crucial and particularities in the “danger signals” and initial production of cytokines may be assumed. Therapeutic use of Interferon alpha is an attempt to foil the survival strategy of *E. multilocularis*.

## ***ECHINOCOCCUS MULTILOCULARIS* IN VITRO: AXENIC CULTIVATION FOR LARVAE, PRIMARY CELL CULTURE AND APPROACHES TO GENETICALLY MANIPULATE THE PARASITE**

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Due to their capacity of causing long-lasting, chronic diseases, parasitic helminths are highly interesting organisms regarding immune manipulation and the evolution of parasitism. To study the underlying host-parasite interaction mechanisms on a molecular basis it is imperative that at least parts of the parasite's life cycles can be reconstituted in vitro under laboratory conditions. Among taenid cestodes, this classical problem of molecular helminthology has, as yet, only been overcome for the larval stage of *Echinococcus multilocularis*. Previous, successful attempts to maintain the *E. multilocularis* metacestode stage in vitro relied on co-cultivation with host feeder cells. Growth and budding of the metacestode vesicles as well as differentiation towards the protoscolex stage have been observed under these conditions. Due to the continuous presence of host cells, these systems are, however, of limited use for studies concerning the effect of defined factors on parasite growth. We have, therefore, established an axenic cultivation system which allows parasite development in the absence of host cells. Using this system, we could show that the *E. multilocularis* metacestode only survives under reducing medium conditions and in the absence of oxygen, indicating that the larvae are highly susceptible towards reactive oxygen species which are formed in the medium during cultivation. The presence of host serum supported metacestode survival for several weeks but proliferation and differentiation was only observed upon addition of conditioned medium; i.e. medium previously incubated with host cells. These results indicated that soluble factors which are released by host cells are absolutely necessary for parasite development. Using the axenic cultivation system we could observe clear effects of host hormones and cytokines on parasite development and gene expression (which are subject of a second abstract in this issue), indicating that the parasite is capable of hormonal cross-communication with the host. The axenic in vitro cultivation system now also allows for an establishment of primary *Echinococcus* cell cultures. Towards this end, we have cultivated *Echinococcus* cells from axenically grown metacestode vesicles (i.e. devoid of contaminating host cells) in the absence of oxygen using hydatid cyst fluid as a growth medium. Under these conditions, *Echinococcus* cells survived and proliferated for more than 12 weeks. Taken together, these data introduce the axenic in vitro cultivation system as a useful tool to study the interaction between mammalian hosts and cestode larvae on the molecular level. On the basis of this cultivation system for intact metacestode vesicles and primary *Echinococcus* cells, we are currently establishing methods for genetic manipulation of the parasite.

## RECENT ADVANCES ON CHARACTERIZATION OF *ECHINOCOCCUS* ANTIGEN B

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Patients with echinococcosis are exposed to variety of parasite-derived antigenic molecules, which may evoke patients' immune response. Antigen B (AgB), initially found in hydatid cyst fluid of *Echinococcus granulosus* is a polymeric lipoprotein of 160 kDa and a highly immunogenic major antigen in echinococcal infection. About 80 to 90% of patients suffering from cystic echinococcosis (CE), caused by the metacestode of *E. granulosus*, and 40% patients suffering from alveolar echinococcosis (AE), caused by *Echinococcus multilocularis* larvae, exhibit IgG antibodies against this antigen. In SDS-PAGE, the *E. granulosus* AgB (EgAgB) disassociates and produces a characteristic ladder-like pattern, consisting of regularly spaced subunits with apparent molecular weights of 8, 16, 24 and 32 kDa and the higher molecular weight subunits were supposed to be derived from the 8-kDa subunit component. The EgAgB shows a high degree of genetic variability and the genes encoding the EmAgB 8-kDa subunit monomers can be grouped into four clades, corresponding to the genes *EgAgB8/1*, *EgAgB8/2*, *EgAgB8/3* and *EgAgB8/4*. It is now evident that the EgAgB in hydatid cyst fluid is at least a polymer of *EgAgB8/1* and *EgAgB8/2* monomers. It has been stated in literature that the recombinant *EgAgB8/2* has better performance in serodiagnosis of CE than the recombinant *EgAgB8/1*. The EgAgB has been characterized as a protease inhibitor with ability to inhibit recruitment of neutrophils and exploit activation of T helper cell by eliciting a non protective Th2 cell response. Recent studies demonstrated that the AgB also exists in metacestode fluid of *E. multilocularis*. Five different but homologous cDNA and their corresponding genomic DNA sequences encoding the EgAgB homologues were isolated from vesicles, protoscoleces and/or immature adult worms of *E. multilocularis* and named as *EmAgB8/1*, *EmAgB8/2*, *EmAgB8/3*, *EmAgB8/4* and *EmAgB8/5*. These genes appeared to be expressed stage specifically in the parasite life cycle. This review focuses on recent advances on molecular biological and immunological characterization of AgB from both of *E. granulosus* and *E. multilocularis*, and stress the stage specific expression of *E. multilocularis* AgB 8-kDa subunit monomers in parasite life cycle.

## USEFULNESS OF SEVERE COMBINED IMMUNODEFICIENCY (SCID) AND INBRED MICE FOR STUDIES OF CYSTICERCOSIS AND ECHINOCOCCOSIS

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Inbred and NOD/Shi-*scid* mice have been used for studying cysticercosis and echinococcosis at Asahikawa Medical College. **1) NOD/Shi-*scid* mice for cysticercosis:** Recent work using three human taeniid species has shown that all these taeniid species develop into fully matured metacestodes (MCs) in the *scid* mice. Comparative analysis of development of MCs of *T. saginata* was carried out using NOD/Shi-*scid*, NOD/Shi-+/+ and C.B-17/Icr-*scid* mice. Males of NOD/Shi-+/+ and C.B-17/Icr-*scid* mice did not harbor any MCs, whereas both sexes of NOD/Shi-*scid* mice harbored fully developed MCs. Although the maximum size of MCs of *T. asiatica* was around 2-3 mm in diameter in pigs (Fan P.C., 1988), it became approximately 10 mm in diameter in NOD/Shi-*scid* mice. Infectivity of MCs of *T. saginata* developed in NOD/Shi-*scid* mice to humans was confirmed by oral inoculation of 12-weeks-old MCs into 4 volunteers. It is expected to do experimental infections in volunteers with a single MC of both *T. saginata* and *T. asiatica* developed in NOD/Shi-*scid* mice in order to get additional information on the taxonomic problem on *T. asiatica*. NOD/Shi-*scid* mice infected with *T. solium* were used for evaluation of the efficacy of oxfendazole especially after MCs had well developed. When NOD/Shi-*scid* mice harboring 3-month-old MCs were treated with oxfendazole for one month, most of MCs including many stunted MCs were found seriously damaged. Histopathological observation of surviving MCs revealed that the parenchyma tissues were seriously destroyed with vacuolations. From these results, NOD/Shi-*scid* mouse may be expected to be a suitable laboratory animal model for screening and evaluation of other drugs. **2) Inbred DBA/2J and NOD/Shi-*scid* mice for echinococcosis:** a) In order to establish murine model of human hepatic echinococcosis, DBA/2J mouse was injected metacestode homogenate into mesenteric vein. According to the method, echinococcal foci could be developed restrictive hepatic site without any metastasis in other organs. b) Evaluation of quantitative measures of development of echinococcal metacestode has been carried out using infected NOD/Shi-+/+ and NOD/Shi-*scid* mice. The weight, size of developed foci and number of vesicles including protoscoleces were 10 to 100 times more in those developed in NOD/Shi-*scid* mice than in NOD/Shi-+/+ mice. Further studies using inbred or *scid* mice are under progress.



## LABORATORY ANIMAL MODELS FOR HUMAN *TAENIA SOLIUM*

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Human beings are the only host of adult *Taenia solium*, thus many aspects of the host-parasite relationship are unknown. We established experimental models in hamsters, gerbils and chinchillas by keeping them immunodepressed along the time that infection lasted. We found important differences in tapeworm development and in host's immune response. Statistical analysis of five infection performed in 136 hamsters indicate that more male become infected but female develop longer tapeworms, cysticerci evagination above 96% and 2-6 mg of steroid favor infection. Parasite antigens and specific antibodies are detected in feces and blood but vary depending of the host's immune status. Hamsters are more permissive hosts than gerbils, since parasites survive for longer times, grow longer and become more developed. The intestinal inflammatory reaction around scolices in immune competent hosts contains mainly eosinophils, goblet cells, plasma cells, and, interestingly, in gerbils, there is an 8 fold increase in mast cells at 2-3 weeks of infection, time when worms are expelled, in contrast hamsters do not develop mastocytosis. The presence of IL-5 in jejunum analyzed in histological sections of biopsies taken at different days post infection (DPI) processed for *in situ* hybridization showed no differences between infected and non-infected hamsters and with the negative control at 4 DPI; at 8 DPI infected animals developed positive reactions that increased until the end of the experiment at 24 DPI. Positive cells were observed first in the muscular layer and in the submucosa and then in the lamina propria and villi. *Chinchilla laniger* is the only experimental definitive host for *T. solium* in which the parasite develops to gravity. Six experiments were performed over 3 years in 76 immunodepressed outbred female chinchillas infected with 352 cysticerci (4 to 6 per animal); 38 tapeworms were recovered, efficiency of infection was 10%, of the tapeworms recovered 14 were gravid (37%), each gravid proglottid had between 10 and 5,000 mature eggs. Viability methods were compared, only trypan blue (54%) and propidium iodide (60%) gave similar results. Only cysticerci with 100% evagination were useful to infect and obtain gravid tapeworms. Along 5 months of infection coproantigens showed irregular distribution, while proglottids in feces, detected since week 9, showed periodic release. Two piglets were infected with 50,000 eggs each; after counting cysticerci recovered at necropsy, 3 months after infection, 0.4% infection rate was obtained, similar to other studies. These experimental models are useful research tools that could still be optimized.

## **EVIDENCE FOR PIG - PIG TRANSMISSION IN CYSTICERCOSIS**

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*Taenia solium* taeniasis/cysticercosis is one of few potentially eradicable infectious diseases and is the target of control programs in several countries. The larval stage of this zoonotic cestode invades the human brain and is responsible for most cases of adult-onset epilepsy in the world. The pig is the natural intermediate host, harboring the larvae or cysticerci. Our current understanding of the life cycle implicates humans as the only definitive host and tapeworm carrier (developing taeniasis), and thus the sole source of infective eggs that are responsible for cysticercosis in both human and pigs through oral fecal transmission. Here we show evidence of an alternative pig-to-pig route of transmission, previously not suspected to exist. In a series of four experiments, naive sentinel pigs were exposed to pigs which had been infected orally with tapeworm segments (containing infective eggs) and moved to a clean environment. Consistently in all four experiments at least one of the sentinel pigs became seropositive or infected with parasite cysts with much lower cyst burdens than did primarily infected animals. Second-hand transmission of *T. solium* eggs could explain the overdispersed pattern of porcine cysticercosis, with few pigs harboring heavy parasite burdens and many more harboring small numbers of parasites. This route of transmission opens new avenues for consideration with respect to control strategies.

## MOLECULAR DISCRIMINATION OF TAENIID CESTODES

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DNA approaches are now being used routinely for accurate identification of *Echinococcus* and *Taenia* species, sub-species and strains and in molecular epidemiological surveys of echinococcosis/taeniasis in different geographical settings and host assemblages. The publication of the complete sequences of the mitochondrial (mt) genomes of *E. granulosus*, *E. multilocularis* and *T. solium*, and the availability of mtDNA sequences for a number of other taeniid genotypes, has provided additional genetic information that can be used for more in depth phylogenetic and taxonomic studies of these parasites. This very rich sequence information has provided a solid molecular basis, along with a range of different biological, epidemiological, biochemical and other molecular-genetic criteria, for revising the taxonomy of the genus *Echinococcus*. This has been a controversial issue for some time. Furthermore, the accumulating genetic data has allowed the more precise delineation of the host and geographic ranges of the genotypes characterized to date, and the development of PCR-based tests for unambiguous identification *Echinococcus* eggs in the faeces of definitive hosts. Molecular phylogenies derived from mtDNA sequence comparisons of geographically distributed samples of *T. solium* provide molecular evidence for two genotypes, one being restricted to Asia, with the other occurring in Africa and America. Whether the two genetic forms of *T. solium* differ in important characteristics such as infectivity or the pathology they cause remains to be determined. As well, minor DNA sequence differences have been reported between isolates of *T. saginata* and Asian *Taenia*. There has been considerable discussion over a number of years regarding the taxonomic position of Asian *Taenia* and whether it should be regarded as a genotype, strain, sub-species or sister species of *T. saginata*. The available molecular genetic data do not support independent species status for Asian *Taenia* and *T. saginata*. What is in agreement is that both taxa are closely related to each other but distantly related to *T. solium*. This is important in public health terms as it predicts that cysticercosis in humans attributable to Asian *Taenia* does not occur, because cysticercosis is unknown in *T. saginata*.

## ***ECHINOCOCCUS SHIQUICUS*, A NEW SPECIES FROM QINGHAI-TIBET PLATEAU REGION OF CHINA: ITS FINDING AND EPIDEMIOLOGICAL IMPLICATION**

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In the Qinghai-Tibet plateau region approximately 4000 meters above sea level, many wild and domestic mammals were found to be involved in the transmission cycles of *Echinococcus* spp. *E. multilocularis* and *E. granulosus* sensu stricto (sheep strain) have been proved to occur there sympatrically. In 1995, we found the unique adult of *Echinococcus* from Tibetan fox but considered it to be a variant of *E. multilocularis*. Afterwards, molecular genetic studies in this region revealed that a hydatid cyst from plateau pika has characteristic mitochondrial DNA sequences which are dissimilar to any published sequences of *Echinococcus* spp. The same sequences were subsequently found in adult worms from Tibetan foxes. Morphological features of both adult and larva are distinguishable from other species of *Echinococcus*. Therefore, it was named as *Echinococcus shiquicus* after its locality of occurrence, Shiqu County. Since then, *E. shiquicus* has been found at other survey spots in this region. Due to natural environment, socio-economic situation, and religious beliefs, Tibetan people live in conditions with a poor standard of hygiene and keep a close contact with wild and domestic animals. The Tibetan plateau of western China is a highly endemic region of both alveolar and cystic echinococcosis. However, human cases caused by *E. shiquicus* have not been confirmed. Further clinical, ecological, epidemiological and biological studies in the plateau are required for better understanding of the transmission dynamics of *Echinococcus* spp.

## EVOLUTION OF THE GENUS *ECHINOCOCCUS* AND PHYLOGEOGRAPHY OF *E. MULTILOCULARIS*

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The taxonomy of *Echinococcus* has been controversial owing to inadequate descriptions and sympatric occurrences of subspecies. The morphospecies of *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli* are generally accepted as valid taxa. However, several strains of *E. granulosus*, which show substantial genetic diversity, have been classified into 10 genotypes (G1-G10). The biological entity of sibling species should be considered in *Echinococcus* and the following taxonomic revision has been made; the G1 (sheep strain) genotype is the prototypical species of *E. granulosus* but the G4 (horse strain) and G5 (cattle strain) genotypes are distinct species of *E. equinus* and *E. ortleppi*, respectively. Only recently, *E. shiquicus* n. sp. has been found from China. In this study, the phylogenetic relationships of 7 species (*E. oligarthrus*, *E. vogeli*, *E. multilocularis*, *E. shiquicus*, *E. equinus*, *E. granulosus* sensu stricto and the G6 genotype of camel strain) are determined by using complete nucleotide sequences of mitochondrial genome. The corresponding sequence of *Taenia solium* was used as an outgroup. In the phylogram constructed by maximum likelihood, *E. oligarthrus* and *E. vogeli*, which are indigenous to the Neotropical region, were positioned near the root of *Echinococcus* speciation with high bootstrap values. The definitive hosts for both of the Neotropical parasites are the descendants of immigrants from North America during the epoch of the Great American Interchange. Excepting the felid parasite *E. oligarthrus*, *Echinococcus* usually uses canid as definitive hosts. The evolution of ancestral canid in North America supports the hypothesis that North America was the cradle of *Echinococcus* ancestor. As concerns *E. multilocularis*, the phylogram of mitochondrial genes well depicted a geographic division into European, Asian, Beringian and North American clades. The low values of pairwise divergence (0.34-0.49%) between the Asian and European clades corresponded to the divergence time of over 100,000 years, when the general estimation of base substitution in mitochondrial DNA (2% divergence per million years) was applied. The discontinuous distribution of *E. multilocularis* in recent Europe supports that the European clade is derived from isolated populations in glacial refugia. In the phylogram, the North American clade was the first to diverge and the Beringian clade followed close behind, suggesting that *E. multilocularis* had been speciated in North America and spread, through Asia, to Europe.

## WHAT IS ASIAN *TAENIA* ?

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Asian *Taenia* is a human tapeworm which was recognized from Taiwan aborigines and subsequently from Asian countries, i.e., Korea, Thailand, Philippines, Indonesia and China. It was originally described as *Taenia asiatica* (Eom and Rim, 1993) based on the morphology in its adult and larval stage. A taxonomic disagreement on whether it is species or subspecies level is mainly due to the morphological similarity of this tapeworm with *T. saginata*, but a sympatric distribution of these two tapeworms is also known in some countries. The life cycle, however, is quite distinct from *T. saginata* in using the pig as intermediate host with its visceral organs such as liver, lung and omentum. A long known mysterious question in Asian countries about the inconsistency between worm ratio and the food eating habit was resolved with this viscerotropic tapeworm. Genotypic polymorphism and intraspecific variation of *T. asiatica* were observed in phylogeographic studies but differential diagnosis between other *Taenia* tapeworm is possible with PCR, multiplex PCR, cytochrome *c* oxidase subunit 1 gene and nucleotides sequence analysis as well as with thymine-base reader analysis. The complete genome of mitochondrial DNA was sequenced recently which may provide a resource for comparative mitochondrial genomics and systematics studies of parasitic cestodes. The genetic divergence of *T. asiatica*, with other human-specific *Taenia* tapeworms, is also used in the study of human evolution. Vaccination in pigs and animal model in scid or immunosuppressed mice were tried. A possibility of human cysticercosis is yet to be studied as well as with a possible cross reaction in serological diagnosis of *T. solium* cysticercosis in endemic areas of these tapeworms.

## PHYLOGENY OF *TAENIA*: DEFINING SPECIES AND ORIGINS OF HUMAN PARASITES

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Phylogeny is fundamental as it constrains explanations about history and forms our foundation for recognizing and diagnosing species. In the absence of such a framework taxonomists historically relied on intuitive processes and personal judgment, embracing a typological view of species, often disregarding otherwise unequivocal biological criteria. Species of *Taenia* are among the most characteristic tapeworms infecting carnivores and humans as definitive hosts and indeed Taeniidae is unique among the Eucestoda in requiring 2 obligate mammalian hosts for transmission; a high percentage (> 80%) of life cycles have been completely elucidated. Until recently there had been no comprehensive attempts at reconstruction of a phylogeny among these important parasites. Such analyses have allowed us to explore the origins and evolution of those species of *Taenia* that occur in humans (*T. saginata*, *T. asiatica*, and *T. solium*) and to understand the ecological and historical processes serving as determinants of biogeography and host-association. These studies supported the independent status of *T. asiatica* as a valid species, in contrast to a diversity of opinions that would subsume it as a subspecies, rather than the sister species, of *T. saginata*. Recognition of a species constitutes a specific and testable hypothesis, and is not an arbitrary decision. Considering macrospecies, a process has been outlined by Brooks and McLennan as follows: 1) Discovery: a systematist describes the species; (2) Evaluation I: do sister species show geographical overlap- are they sympatric or allopatric (use phylogeny + geographical distributions)? (3) Evaluation II: Are sister species reproductively isolated based on information from natural history, ecology and reproductive biology (experimental evidence, cross-breeding, genetic, morphological, behavioral, host associations, transmission, life-history, biogeography)? Further we must view species in the context of microevolutionary and macroevolutionary processes. For instance, microspecies are defined in ecological time and involve populations and contemporary process that are potentially reversible. In contrast, macrospecies as exemplified by *T. saginata* and *T. asiatica* are divergent lineages resulting from processes in evolutionary time where an ancestor has undergone a permanent split that is non-reversible. Applying these criteria in evaluation of *T. saginata* and *T. asiatica*, it becomes clear that these represent independent historical lineages in space and time, having separated from a common ancestor near 0.78 to 1.71 MYBP in Africa, or Eurasia. In ecological time, the sympatry and reproductive isolation evident for *T. saginata* and *T. asiatica* in China and perhaps other regions of SE Asia further serve to validate these taeniids.

## **ALVEOLAR ECHINOCOCCOSIS IN CHINA: INTERNATIONAL COLLABORATION AND CONTRIBUTIONS TO EPIDEMIOLOGICAL RESEARCH (1994 2004)**

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Prior to the 1990's relatively little was known about the epidemiology of human alveolar echinococcosis (AE) and the transmission of *Echinococcus multilocularis* in the endemic region of western China. Pilot surveys for human AE in Han Chinese peasant communities in south Gansu Province, based on ultrasound scanning (US) and serological confirmation, confirmed high average prevalence (4%, village range 0-15.8%). Subsequent surveys in mixed Han/Hui agricultural areas of south Ningxia Region determined an AE prevalence of 1.5% (village range 0-8.1%). In Tibetan communities in northwest Sichuan higher average AE prevalence was found (6.5%, township range, 0-14.3%). Human incidence of AE in the far northwest Xinjiang Region varied from 0-3.9 per 100,000 (mean 0.6) with highest rates in Mongol and Kazakh ethnic groups. For US detected cases average age was 43.1 years in Shiqu county (Sichuan) and 38.7 years in Zhang/Puma counties (Gansu), with females at higher risk in both regions. Family clustering of cases also occurred in several communities. Explanations for high AE prevalences in China continue to be investigated. Despite major differences in ethnic, socio-cultural, livestock densities, agricultural activities and geo-environmental features between counties and regions, however, key significant risk factors for human AE were history of dog ownership and landscape characteristics associated with scrubland and/or grassland. Other factors included, hygiene parameters, livestock ownership and husbandary practices. Ongoing research includes the role of ecosystem disturbance on the transmission ecology of the parasite, and development of spacio-deterministic models to describe endemicity, to forecast disease risk and to consider prevention/control options.



## TRANSMISSION ECOLOGY OF *ECHINOCOCCUS* IN CHINA: ADVANCES AND HOT QUESTIONS

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In continental China *Echinococcus multilocularis* (Em) transmission intensity is spatially heterogeneous and unstable. This instability makes transmission responsive to fox/dog dosing control campaigns and variations in host population densities. Parasite extinction may occur as a consequence of landscape/land use change (e.g. Zhang-Puma area of southern Gansu, Northern Liu Pan Shan, Ningxia). By contrast, intensive and more stable transmission occurs in some areas of the Tibetan plateau due to sustainable high densities of small mammal intermediate host populations, the existence of two species of fox definitive hosts, and the extremely high number of dogs in and around villages with easy access to small mammal colonies. Yak herd management and subsequent grazing patterns may be an important factor impacting both the composition of small mammal communities and the population dynamics of intermediate host-species. Using data collected from comprehensive surveys in China and Europe as evidence, this paper sets out four concepts of transmission addressed by the following questions:

- 1) Do short-term studies within localized (sub-county) areas provide sufficient information to understand the processes leading to stable transmission of Em?
- 2) If no, how many small mammal communities and landscape habitats may be significantly involved in transmission?
- 3) Thus, what is the spatial extent of areas within which meta-stability operates and which areas can subsequently be considered stable foci ?
- 4) To which geographical range can the parasite be spread by foxes and the dog trade, thus can the likely meta-stable foci of the Tibetan plateau be a source of infection to transitory favorable remote areas of the spurs of the plateau (e.g. Southern Gansu) or even of more remote ranges (e.g. Liu Pan Shan)?

## **SPATIAL MODELLING AND ECOLOGY OF *ECHINOCOCCUS MULTILOCULARIS* TRANSMISSION IN CENTRAL ASIA**

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Transmission of *Echinococcus multilocularis* to humans occurs by accidental ingestion of eggs either indirectly through contaminated vegetation, or directly through contact with an infected canid. Our work in China, and more recently in Kazakhstan, has suggested that the most likely transmission mechanism in these regions is *via* domestic dogs which are allowed to roam freely and hunt (infected) small mammals within areas close to villages or tented pastures. This assertion has led to the hypothesis that there is a landscape control on transmission risk since the proximity of suitable habitat for susceptible small mammals appears to be key. We have tested this hypothesis in a number of endemic areas in China, notably south Gansu and Ningxia Provinces and most recently in the Tibetan region of western Sichuan Province. The fundamental landscape control is its effect at a regional scale on small mammal species assemblages (susceptible species are not ubiquitous) and, at a local scale, the spatial distributions of small mammal populations. To date we have examined relationships between landscape composition and patterns of human infection, and landscape and small mammal distributions. In current work we are examining the relationships between landscape and dog infection rates. The key tool to characterize landscape is satellite remote sensing and these data are used as inputs to drive spatial models of transmission risk. This paper reviews the progress that has been made so far in spatial modeling of the ecology of *E. multilocularis*, outlines current research issues, and describes a framework for building a spatial-temporal model of transmission risk.

## **PREVALENCE, DIAGNOSIS AND TREATMENT OF HUMAN ECHINOCOCCOSIS IN CHINA**

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Human echinococcosis is still a major public health problem, especially in northwestern, northern and central parts of China, such as Xinjiang, Gansu, Tibet, Inner Mongolia, Qinghai, Ningxia and Sichuan. Based on a China CDC meeting in Urumqi and Dunhuang, approximately 1,000,000 hydatid patients have been recorded in hospital data with involved 20,000,000 population and 80,000,000 RMB loss by this endemic disease, although there were still no official statistical hydatid incidence in China. Only in the clinical division of the First Teaching Hospital, Xinjiang Hydatid Clinical Research Institute (XHCRI), 5,434 patients have been recorded as 3,643 (67.04%) cases in liver, 1,153 (21.22%) in lungs, 125 (2.30%) in the abdominal/pelvic cavity, 108 (1.99%) in brain, and 531 (7.45%) in other organs including kidney, bone, spleen, pancreas and heart. A “fast immunodiagnostic kit” for clinical confirmation and differentiation of human cystic and alveolar echinococcosis with over ninety percent of sensitivity and specificity, which can be conveniently economically used in both clinic and mass screening. (1) Surgical treatment: Surgery for human cystic echinococcosis: (i) intact pericystectomy for hepatic CE, (ii) punctured endocystectomy for hepatic CE, and (iii) PAIR. (2) Surgery for AE cases: (i) radical hepatectomy, (ii) special approach. Liver transplantation for late stage AE patient. Besançon Central Hospital (France). The first AE patient in China was successfully transplanted by side-to-side piggyback procedures on 27th, December 2000 in XHCRI and the following was also successfully done, in Sichuan and Chongqing the Liver Transplant Centres. Chemotherapy: Liposomal formulation strongly changed free albendazole in tissue distribution in rodents (mice, gerbils and cotton rats). Targeting function was experimentally confirmed in liver, lungs, spleen etc., which showed L-ABZ has significant organ selection and high bioavailability. Initial clinical trial with a total of 71 hydatid patients (12 cases for AE and 59 for CE), had a positive effect with minor side effects.

## SECOND NATIONAL SURVEY DATA OF ECHINOCOCCOSIS AND CYSTICERCOSIS IN CHINA

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In order to understand and analyze the current epidemiological situation and transmission trends of major parasitic diseases in China, the Ministry of Health organized a survey of current situation of major human parasitic diseases in 31 provinces/autonomous regions/municipalities (P/A/M) in China (Taiwan, Hongkong and Macao not included) since 2001. A total of 356,629 persons from 31 P/A/M were investigated, the infection rate of *Taenia* spp. was 0.28%. Among all the P/A/M investigated, the highest standardized rate of *Taenia* infection was in Tibet (21.08%), followed by Xinjiang (0.74%), and no *Taenia* spp. infection was found in other 19 P/A/M. Among 96,008 persons examined by cysticercosis serological test in the whole country, 553 persons were serologically positive, and the serological positive rate was 0.58%. Among the 39,826 persons examined by echinococcosis serological test in 12 provinces/autonomous regions, the serological positive rate was 12.04%. Among the 34,486 persons were examined by B-mode ultrasonography in 12 P/A/M, 374 echinococcosis cases were detected and the prevalence of echinococcosis was 1.08%.

## CLINICAL ALVEOLAR ECHINOCOCCOSIS AND TREATMENT

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The Hokkaido government confirmed a total of 462 cases of alveolar echinococcosis from 1936 up to March 2005 in Hokkaido. During the same time, about 30 cases were reported in Honshu. In recent years, 20-30 new patients have been reported annually in various parts of Hokkaido, and the incidence of alveolar echinococcosis has been increasing slightly. In the First Department of Surgery at Hokkaido University Hospital, the first patient with alveolar echinococcosis underwent surgery in 1936. The "International Symposium on Alveolar Echinococcosis – Strategy for eradication of alveolar echinococcosis of the liver" held in Sapporo in February 1995 discussed the surgical outcomes for 156 patients who had been treated at Hokkaido University Hospital. All 71 patients who underwent complete resection remained alive without recurrence for up to 20 years. When diagnosis was late and the disease was highly advanced, exploratory laparotomy, marsupialization or biliary drainage was performed instead of resection on 38 patients (24%). Outcomes for these patients were poor. The use of albendazole (ABZ, trade name: Eskazole) was approved in Japan in 1994, and after the Sapporo Symposium, surgery has been successfully performed on 89 patients, all of whom remain alive and without recurrence as of the time of writing. ABZ has been used as an auxiliary agent in about 50% of patients for about 1 year. Now, a total of 236 patients have undergone surgery. Over the last decade or so, 3 patients developed advanced alveolar echinococcosis resulting in pericardial infiltration, inferior vena cava infiltration or hepatopulmonary fistula, and the lesion was excised with the inferior vena cava or right lower pulmonary lobe. With ABZ administration, clinical course for all of these patients has been favorable after surgery. In patients with multiple liver lesions, metastasis or recurrence, ABZ has been administered for up to 10 years, and growth arrest and reduction of the lesions have been confirmed. MRI is useful, and a solid area is hypointense on T1-weighted imaging and hyper- (36%), iso- (27%) or hypointense (38%) on T2-weighted imaging. Most lesions are not detectable by contrast radiography, and small cystic lesions can be more clearly detected by CT when compared to T2-weighted MRI. Calcification is undetected (Kodama et al., Radiology, 2002). More than half of the patients were ranchers and farmers. Construction workers tend to display more advanced disease. The foxes move into urban areas to affect students and housewives, and this represents a serious problem.

## CONTROL OPTIONS FOR *ECHINOCOCCUS MULTILOCULARIS* IN JAPAN FROM THE VETERINARY POINT OF VIEW

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*Echinococcus multilocularis* is distributed all over Hokkaido, the northern island of Japan. The prevalence of foxes has been around 40% in the last decade and the habitat of foxes has been getting closer or overlapping to that of human, producing potential risk of infection to humans and companion animals. For the contribution to control of echinococcosis from the veterinary point, two trials of anthelmintic distribution against wild foxes were conducted in Hokkaido. In Koshimizu, a flat rural town, baits containing praziquantel were distributed around fox breeding dens, along most of the roads in the town or at the cross sections of the roads and windbreak forests. Fox feces were collected in the study area, and coproantigen and fecal eggs were examined. The egg excretion from foxes was reduced rapidly after the bait distribution, however, coproantigen excretion from foxes remained relatively high level for a year and then decreased. It is suggested that foxes were easily reinfected after dewormed in the first year, but the chance of reinfection was reduced in the following year due to the decrease of infected rodents. In Otaru, a city surrounding by mountainous area, baits were distributed along the roads in the city. In this trial, tetracycline was put into the anthelmintic baits so that consumption of the baits by foxes could be detected by the examination of the tetracycline line in the bone and canine teeth. The prevalence of foxes was evaluated by the necropsy of the animals captured by local hunters. Majority of the foxes having tetracycline line did not harbor the parasite, indicating foxes consuming the baits were effectively dewormed. In parallel to this, a survey of companion dogs has been conducted using a diagnostic system composed of coproantigen, fecal egg and DNA detection methods. So far, 15 dogs were determined infected out of 3,688 dogs examined, among which 8 dogs were confirmed excreting *E. multilocularis* eggs. Most of the infected dogs were kept free, showed interest to rodents or were eager to pick up (and eat) found materials during a walk. In addition, a dog which was transported from Hokkaido to the main island of Japan was found excreting *E. multilocularis* eggs. The results roused the public recognition of dog infections, which in turn lead to the modification of a Japanese law for infectious diseases and to the enforcement of a national reporting system by veterinarians for dogs infected with *E. multilocularis*.

# MATHEMATICAL MODELING OF *ECHINOCOCCUS MULTILOCULARIS* TRANSMISSION IN JAPAN

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Human alveolar echinococcosis (HAE) has spread throughout the mainland of Hokkaido, making it desirable to design effective control strategies against HAE. It is difficult to elucidate the source of infections due to the long incubation period. A mathematical model for *Echinococcus multilocularis* transmission would be useful for estimating its prevalence. We proposed a model of the *E. multilocularis* transmission cycle between the major definitive and the intermediate hosts in Hokkaido, Japan in which the function of control measures against *E. multilocularis* was incorporated. We carried out simulations of the model to investigate whether *E. multilocularis* maintains its transmission cycle in two wild animal hosts in Hokkaido. Since the dynamics of both the definitive and intermediate host populations show marked seasonal variations that influence *E. multilocularis* transmission, a model that describes the transmission of *E. multilocularis* quantitatively needs to include a host population dynamic component. The major definitive hosts, red foxes, are infected with *E. multilocularis* by preying on infected voles (major intermediate hosts). Thus, in the model, the function of transmission from voles to foxes was designed based on fox feeding habits. We also introduced a hazard index to assess the risk to the human population of being infected with HAE on the assumption that the risk would depend on the abundance of parasite eggs in the environment. The ecological and epidemiological parameters used in the model were based on the findings of field surveys in Hokkaido. Simulation clarified the seasonal transition in the prevalence of *E. multilocularis* in the fox population under both climatic and ecological influences, although little was previously known about this transition due to the lack of seasonal data. The model also supported a fair decline in the hazard index by distribution of baits containing praziquantel. Simulations could also be helpful in estimating the usefulness of bait distribution programs. Further follow-up study based on field data is desirable.

## CLINICAL ASPECTS OF NEUROCYSTICERCOSIS

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The clinical features of neurocysticercosis (NCC) largely depend on the number, type, size, localization and stage of development of cysticerci, as well as on the host immune response against the parasite. There are no pathognomonic features or a typical NCC syndrome. When NCC is intraparenchymal, it is usually associated with a good prognosis. Frequently, patients with few intraparenchymal cysts remain asymptomatic, although some patients develop seizures. On the other hand, in patients with massive cerebral infection, uncontrolled seizures and cognitive deficit may develop. Seizures are widely reported to be the most common symptom, occurring in 70-90% of patients and NCC is considered to be the main cause of late-onset epilepsy in endemic areas. In most cases, partial seizures, with or without secondary generalization, predominate. An increase in the frequency of seizures has been reported to coincide with the death of the cysticercus. In many patients, as the cysticercus calcifies, seizures tend to become less frequent although patients usually require continuous treatment with antiepileptic drugs. When cysticerci lodge within the ventricular system, life-threatening acute intracranial hypertension secondary to hydrocephalus may develop. Cysts in the subarachnoid space may invade the Sylvian fissure and grow to large sizes (giant cysts) causing intracranial hypertension with hemiparesis, partial seizures or other focal neurological signs. Racemose cysts in the basal cisterns can cause an intense inflammatory reaction, fibrosis and progressive thickening of the leptomeninges at the base of the brain. In approximately 60% of the cases, there is an obstruction of the CSF circulation, resulting in hydrocephalus and intracranial hypertension. Signs of meningitis, cranial nerve palsy, chiasmatic syndrome, cerebellopontine-angle syndrome and cerebral infarcts secondary to vasculitis may also develop. When hydrocephalus secondary to cysticercotic meningitis is present, the mortality rate is high (50%) and most patients die within 2 years after CSF shunting. Therefore, ventricular and basal cisternal locations are considered to be malignant forms of NCC. Intracranial hypertension also occurs in patients with cysticercal encephalitis, which occurs as the result of a massive infection of the brain parenchyma inducing an intense immune response and diffuse brain edema. Involvement of the spinal cord is rare, accounting for 1-5% of all cases of NCC; the clinical manifestations usually include progressive paraparesis with a sensory level and sphincter disturbances.



## IMAGING ASPECTS AND NONSURGICAL INTERVENTIONAL TREATMENT IN HUMAN ALVEOLAR ECHINOCOCCOSIS

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Alveolar echinococcosis (AE) of the liver is characterized by a multivesicular structure surrounded by an extensive fibro-inflammatory host reaction. The lesions behave like a slow-growing liver cancer, without sharp limits between the parasitic tissue and the liver parenchyma. Invasion of biliary and vascular walls is another hallmark of this severe disease. Moreover, poor vascularization of the parasitic mass often leads to necrosis in the central part of the lesion. This explains why liver abscess due to bacterial super-infection of the necrotic area may occur in this disease. Currently, a range of imaging techniques can be used at the different steps of AE management. For diagnosis, ultrasonography (US) associated with colour doppler, remains the first and most commonly used examination. The typical AE lesion is a heterogeneous tumour, with a juxtaposition of hypoechogenic and hyperechogenic foci and irregular outlines. Vascular involvement, hilar invasion and its effect on the intrahepatic biliary tree can also be precisely analysed by US. However, physicians in endemic areas must be aware of atypical ultrasonic aspects of AE such as huge pseudocystic lesions related to massive necrosis and small hyperechogenic nodular AE foci simulating liver haemangioma. For a more precise disease evaluation, to guide the surgical strategy, CT-scan, Magnetic Resonance Imaging (MRI), including cholangio-MRI are of great assistance as they provide complementary information. Precision on the number, size and localisations of AE foci is given by CT-scan which is also able to show the typical calcifications. MR imaging plays a major role in delineating the extension of the parasitic tumour, especially to the diaphragm and abdominal structures and/or organs, to intrahepatic vessels, bile ducts, and to the vena cava. Moreover, it can give a precise analysis of the different components of the parasitic mass. MRI is the only technique able to identify the pathognomic multilocular aspect of AE lesions, but it fails to recognize calcifications. For the follow-up of patients having had a curative surgical resection, MRI seems to be more sensitive than CT-scan in disclosing recurrent AE foci. More recently, Positive-Emission Tomography (PET) using [<sup>18</sup>F] fluoro-deoxyglucose has been developed for the follow-up of inoperable AE patients under long-term benzimidazole (BZM) therapy. This approach appears very promising to assess inflammatory activity and thereby to indirectly depict parasitic activity, especially when it is combined with CT-scan using image fusion. Non surgical interventional procedures, mainly percutaneous biliary and/or centro-parasitic abscess drainages, currently play a major role in the management of incurable AE patients and have largely contributed to the improvement of survival in this situation over the past 20 years. It may be used as a bridge before curative surgery in symptomatic patients presenting a life-threatening bacterial and/or fungal infection. They are also very useful in inoperable patients to overcome similar infectious episodes.

## **CLINICAL STUDIES WITH NEW DRUGS FOR ECHINOCOCCOSIS**

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Chemotherapy is one of the main components for the treatment of alveolar and cystic echinococcosis. Benzimidazoles are on the market since 30 years, and are used widely according to the recommendations of the 1996 WHO guidelines for treatment of cystic and alveolar echinococcosis (Bull WHO 1996; 74:231-242). However, none of the two drugs (albendazole or mebendazole) would satisfy today the requirements for an approval by the relevant agencies, such as the Food and Drug Administration (FDA) or the European Agency for the Evaluation of Medicinal Products (EMA). The situation might be comparable to that of a series of anti-infective agents for well established indications, i.e. penicillin and streptomycin for the infectious endocarditis. For echinococcosis, new drugs are in the pipeline, and some of them have already undergone preclinical testing. Advances have been made in the definition of outcomes for clinical trials in alveolar and in cystic echinococcosis. Thus, phase II and phase III studies with new active ingredients are needed to assess short- and long-term safety as well as the therapeutic value. The basic requirements for comparative drug testing are provided in the Note for Guidance on Good Clinical Practice (GCP), and are based upon the rules and regulations by International Conference on Harmonization (ICH) and the Clinical Trial Directive (Directive 2001/20/EC) of the European Commission. The application of these standards to new drug testing for echinococcosis will be reviewed.

## COPROANTIGENS IN TAENIASIS / ECHINOCOCCOSIS

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Historically the diagnosis of intestinal taeniid cestodes by traditional parasitological techniques was limited in terms of both sensitivity and specificity. Eggs from different taeniid species appear identical under the light microscope and are often absent from faeces during infection. These factors resulted in problems in the collection of epidemiological data on these parasites. The application of modern immunodiagnostic or molecular diagnostic techniques has improved this situation. One approach that has shown particular promise is the detection of parasite specific antigens in faeces (coproantigens). This approach has now been successfully applied to diagnosis of both *Echinococcus* and *Taenia* species cestodes in humans and dogs. Based on either murine monoclonal or rabbit polyclonal antibodies to adult worm somatic or excretory secretory products these tests have the following common characteristics; they are genus specific, overall specificity is very high (>95%), antigen can be detected in faeces prior to patency and levels of antigen appear independent of egg output, antigen can be detected in formalin fixed faecal samples and antigen levels drop rapidly following successful treatment of the parasite. With the available *Taenia* tests most work has been done on *T.solium*. With this species coproantigen based microplate ELISA detected between two and three times as many worm carriers as microscopy. For *Echinococcus* species there is a positive correlation between test sensitivity and worm burden with a reliable threshold level for the test to be able to detect infection burdens >50 worms. Work on characterising taeniid coproantigens in order to further improve the tests is ongoing. The antigens appear to be largely of high molecular weight (>150kDa), heavily glycosylated and with carbohydrate moieties contributing substantially to the levels of antigen detected in faeces. Other areas that may improve the application of these tests include improving test format to increase ease of application and the establishment of reference laboratories to provide suitable reagents and training to allow use of these tests in projects globally. Application of the existing coproantigen tests in epidemiological and control programmes for *Echinococcus* and *Taenia* species infection has contributed to an improved understanding of these important zoonotic cestodes.

## COPRO-DNA TESTS FOR DIAGNOSIS OF ANIMAL TAENIID CESTODES

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Cestode DNA excreted with eggs, proglottids or parasite cells can be detected from faeces after amplification by the polymerase chain reaction (PCR). However, due to the presence of PCR-inhibitory substances in faecal material, either a laborious DNA purification procedure is indispensable or only a limited amount of faeces (around 200 mg) can be processed as recommended by manufacturers of commercially available DNA isolation kits. One approach to overcome these limitations is to perform an initial step of concentrating helminth eggs by a combination of sequential sieving and an in-between step of flotation in zinc chloride solution (6). Hence, helminth eggs can be concentrated from large sample volumes in a few  $\mu$ l of fluid and detected by means of an inverted microscope in a closed tube. Only samples containing taeniid eggs, which morphologically cannot further be distinguished, need to be further investigated by PCR after DNA extraction using a simplified protocol. Two different genes have so far been targeted in diagnostic PCR for the detection of intestinal *Echinococcus multilocularis* infection, the U1 snRNA gene (2) and the mt 12S rRNA (5, 8), and these tests are routinely being used by many groups (3). It is only recently that PCR has been evaluated for diagnosis of *Echinococcus granulosus* in faecal samples targeting a repetitive sequence (1) or a defined mitochondrial sequence (4, 7). All these assays focus on the detection of *E. granulosus* 'sheep strain' (G1). In addition, Dinkel and colleagues (4) describe a pair of PCR primers that detects the G5 ('cattle'), G6 ('camel') and G7 ('pig') strains with the possibility to subsequently identify the respective strain by performing a second PCR employing strain-specific primers (semi-nested PCR). No PCR assays are available for identification of intestinal *Taenia* spp. of animals. Choosing a broader approach, we are currently developing multiplex PCRs for the discrimination of taeniid eggs. Depending on the question addressed (diagnosis in individual definitive hosts, contamination of the environment and assessing of the infection risks for humans, assessing the effects of control programmes) and on the geographic area, various combinations of primers are being evaluated for their power to differentiate *Taenia* from *E. granulosus* and *E. multilocularis* as well as for the identification of important *Taenia* species.

(1) Abbasi et al., 2003. Am J Trop Med Hyg 69:324. (2) Bretagne et al., 1993. Parasitology 106:193. (3) Deplazes et al., 2003. Parasitology 127:S53. (4) Dinkel et al., 2004. Int J Parasitol 34:645. (5) Dinkel et al., 1998. J Clin Microbiol 36:1871. (6) Mathis et al., 1996. J Helminthol 70:219. (7) Stefanic et al., 2004. Parasitol Res 92:347. (8) Stieger et al., 2002. Parasitology 124:631.

## MITOCHONDRIAL DNA DIAGNOSIS FOR TAENIASIS AND CYSTICERCOSIS

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The differentiation of *Taenia* spp. causing taeniasis and cysticercosis in humans has been routinely performed on the basis of morphology of the parasites. However, it is not always easy to identify or diagnose accurately them due to the morphological similarity of the parasites, particularly *Taenia saginata* and *Taenia asiatica*, or due to the degree of degeneration or calcification of the parasite tissues found in the histopathological preparations. Moreover, it is also impossible to differentiate morphologically two genotypes of *T. solium*, i.e., Asian and American/African genotypes which are distributed in Asia, and Latin America and Africa, respectively. Therefore, in order to overcome limitations in identification of human taeniid cestodes based on the morphology, mitochondrial DNA diagnosis has been developed: PCR-restriction fragment length polymorphism (RFLP), base excision sequence scanning thymine-base reader analysis (BESS T-base) and multiplex PCR. The PCR-based method has also been applied to genotyping of the *T. solium* cysticercus found in the histopathological specimens from cysticercosis patients. In the present symposium, mitochondrial DNA diagnosis using cytochrome *c* oxidase subunit 1 and cytochrome *b* genes will be introduced. The multiplex PCR using *Taenia* spp. DNA in fecal samples for detection of tapeworm carriers will be also presented.

## RECOMBINANT ANTIGENS FOR SERODIAGNOSIS OF CYSTICERCOSIS AND ECHINOCOCCOSIS

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Diagnosis of cysticercosis/echinococcosis is primarily based on imaging techniques, including computed tomography and magnetic resonance imaging. These imaging techniques are sometimes limited by the small size of visualized lesions and atypical images, which are difficult to distinguish from abscesses or neoplasms. Therefore, efforts have been directed toward identification and characterization of specific antigens of parasites for development of serodiagnostic method that can detect specific antibody.

For cysticercosis the glycoproteins of 10-26 kDa in cyst fluid of *Taenia solium* have been widely accepted for serodiagnosis purpose. The glycoproteins consist of a very closely related family of 7-kDa proteins. We identified four genes (designated Ag1, Ag1V1, Ag2 and Ag2V1) encoding 7- and 10-kDa polypeptides. Based on the antigenicities of these clones, Ag1V1 and Ag2 were chosen as ELISA antigens and the Ag1V1/Ag2 chimeric protein was expressed. The Ag1V1/Ag2 chimeric protein showed the similar sensitivity and specificity as the native glycoproteins.

For alveolar echinococcosis the 65-kDa protein of *Echinococcus multilocularis* protoscolices, which was described by several groups, and Em18 (18-kDa protein under reducing condition) reported by us have been considered as serodiagnostic antigens. The sensitivity and specificity of Em18 are very compatible to those of the recombinant 65-kDa protein. Recently, we demonstrated that Em18 is the proteolytic product of the 65-kDa protein following the action by cysteine proteinases. From the information of N-terminal amino acid sequences, molecular size and isoelectric point of Em18, recombinant Em18 (<sup>349</sup>K to <sup>508</sup>K of the 65-kDa protein, RecEm18) was expressed and evaluated for serodiagnostic value. RecEm18 has the potential for use in the differential serodiagnosis of alveolar echinococcosis.

In this symposium, we will present recent advances in serodiagnosis for cysticercosis/echinococcosis in addition to our recent works.

## ***ECHINOCOCCUS MULTILOCULARIS* SIGNAL TRANSDUCTION SYSTEMS: THEIR ROLE IN PARASITE DEVELOPMENT AND HOST-PARASITE INTERPLAY**

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As bilaterian animals, helminth parasites share a large pool of genetic heritage with mammals. One of our aims is to understand whether, and to what extent, this plays a role in long-term persistence of helminths within their mammalian hosts. As a model organism for these investigations, we have chosen the fox-tapeworm *Echinococcus multilocularis*. Our experimental approach mainly bases on an axenic *in vitro* cultivation system for the *Echinococcus* metacestode stage which we have developed to reconstitute the situation at the host liver during the infection (see additional abstract in this issue). Using this *in vitro* system, we could show that two host hormones/growth-factors, insulin and bone morphogenetic protein 2 (BMP2), a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) family of cytokines, significantly stimulate parasite survival and growth. As possible mediators of these effects, we have characterized the parasite surface receptors EmIR, a member of the insulin receptor family of tyrosine kinases, and several members of the transforming growth factor  $\beta$  receptor family (type I and type II) of serine/threonine kinases. EmIR is located at the surface of metacestode vesicles, facing host tissue, and is phosphorylated in response to exogenously added human insulin. At least one of the identified receptors of the TGF- $\beta$  family, EmTR1, a member of the ALK1 subfamily of TGF- $\beta$  receptors, is able to functionally interact with human BMP2 and phosphorylates the downstream TGF- $\beta$  signaling factor EmSmadB *in vitro*. EmIR and EmTR1 are, therefore, promising candidate receptors that could mediate the effects of host insulin and BMP2 on parasite development. In addition to insulin- and TGF- $\beta$  signaling mechanisms, we also identified a member of the epidermal growth factor (EGF) family of cytokines, Egfd, in the parasite as well as a corresponding receptor tyrosine kinase, EmER and several downstream signaling components of the mitogen activated protein kinase (MAPK) cascade. All these systems share considerable structural and functional homologies with the respective signaling mechanisms in mammals. Upon incubation with host cells, the expression of Egfd is significantly upregulated in the metacestode indicating a role of EGF-signaling in parasite development and, possibly, in host-parasite interaction. Taken together, these data show that *E. multilocularis* expresses cell-cell-communication systems with considerable homology to those of mammalian hosts and that these evolutionary conserved molecules may play an important role in host-parasite interactions during alveolar echinococcosis. The significance of these interactions will have to be clarified in further experiments using the axenic *in vitro* system and, in particular, *in vivo* infection models.

## **IDENTIFICATION AND INTRA-SPECIFIC VARIABILITY ANALYSIS OF SECRETED AND MEMBRANE BOUND PROTEINS FROM *ECHINOCOCCUS GRANULOSUS***

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The species *Echinococcus granulosus* comprises a number of genetic variants or strains that differ in biological features, such as intermediate host specificity, developmental rate and infectivity to humans. It is very important to analyse the existence of associated genetic variation in secreted and membrane bound (S/M) protein coding genes, since this may improve knowledge on strategies of parasite survival in the specific host, with practical consequences in immunodiagnosis and immunoprophylaxis. We used PCR- Single strand conformation polymorphism (SSCP) analysis followed by sequencing to address the genetic variation of the major secreted antigen, the antigen B, and a number of S/M proteins identified by the signal sequence trap technique. Forty two AgB-related genomic sequences were isolated from five *E. granulosus* strains and clustered in five genes (B1 to 5) and one pseudogene by maximum parsimony analysis. Differential distribution and frequency of AgB variants among strains were found. The level of nucleotide and amino acid variation observed was higher than the reported so far for coding genes of other helminth parasites. Reverse transcription (RT)-PCR analysis showed that the expression of AgB variants also differed between strains. The signal sequence trap technique was applied in order to identify other genes coding for S/M proteins. Some of the deduced amino acid sequences of the positive clones showed significant similarity with amino acid and Krebs cycle intermediates transporters, presenilins, vacuolar protein sorter proteins, an *E. granulosus* EST coding for a secreted protein, and some were completely novel. Intra-specific variability studies by PCR –SSCP technique confirmed by sequencing, showed that many of the identified S/M protein coding genes were variable between strains. These results suggest that variation in proteins exposed to the host is a more widespread phenomenon in *E. granulosus* than previously appreciated. We suggest that this variability should be taken into account for the rational design of diagnosis systems of cystic hydatid disease. The diagnostic value of some of the protein variants detected in *E. granulosus* strains could be determined through inter-laboratory studies as the recently done by the South American Network for Hydatid Serology.



## **POLYMORPHISM OF MITOCHONDRIAL AND MICROSATELLITE DNAs OF *TAENIA SOLIUM***

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*Taenia solium* taeniasis is a major risk factor for neurocysticercosis and the leading cause of epilepsy in endemic countries in Southeast Asia and South America. Examining the genetic structure of tapeworms in communities (Peru) can be applied to the epidemiology of *T. solium* transmission. Determining genetic variation within and between populations provides insight into future evolutionary changes and will help determine human exposure and transmission patterns. DNA polymorphism has been detected between a panel of Peruvian *T. solium* isolates using PCR-RFLP, microsatellites and mitochondrial(mtDNA). PCR-RFLP of the gene region cytochrome *b* detected 10 alleles showing polymorphisms amongst the isolates. Trinucleotide 'cac' repeat loci detected allelic differentiation. Single Nucleotide Polymorphism from 4 mtDNA sequences identified point mutations using SNaPshot analysis. These markers will be applied to analyse the molecular epidemiology of *T. solium* in endemic communities.

## THE *TAENIA SOLIUM* GENOME PROJECT

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We have constituted a consortium of key laboratories at the National Autonomous University of Mexico to carry out a genomic project for *Taenia solium*. This project will provide powerful resources for the study of taeniasis/cysticercosis and will contribute to the advent of genomics for cestode parasites. Our project is planned in two consecutive stages. The first stage is being carried out to determine some basic parameters of the *T. solium* genome: size, karyotype, gene density, etc. In the second stage a full blown genome project will be launched. We have estimated the genome size by two different approaches: cytofluorometry on isolated cyton nuclei, as well as a probabilistic calculation on the coding and repeat density of the genome based on 3,000 sequenced genomic clones, resulting in size estimates of 270 and 251 Mb, respectively. In terms of sequencing, our goal for the first stage is to characterize several thousand EST's (from adult worm and cysticerci cDNA libraries) and genomic clones. Results obtained so far show that only about 30% of the *T. solium* coding sequences have known homologues. Many of the best hits are found with mammalian genes, particularly with humans. However, 1.5% of the hits lack homologues in humans, making these genes immediate candidates for investigation on pharmaco-therapy, diagnostics and vaccination. As expected for a metazoan eukaryote, most *T. solium* EST's are related to gene regulation, and signal transduction. Other EST's encode for housekeeping function, metabolism, cell division, cytoskeleton, proteases, vacuolar transport, hormone response, and extracellular matrix activities. The coding density for the *T. solium* genome appears relatively high: 7% of all nucleotides are involved in protein coding. Intron size and number within genes appears unexceptional. Preliminary results also suggest that the genome is not highly repetitive. As the project has been conceived as an assemblage of human and physical resources in parasitology, molecular biology and bioinformatics, all data and materials are immediately available to members of the consortium.

## TAENIASIS AND CYSTICERCOSIS IN BALI AND NORTH SUMATRA, INDONESIA

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It has been reported that three human taeniid species are distributed in Indonesia: *Taenia solium*, *Taenia asiatica* and *Taenia saginata*. *T. asiatica* is well known in North Sumatra, especially in Samosir Island in the Lake Toba. *T. solium* and *T. saginata* are known from Bali. *T. solium* is the most serious public health issue in Papua (former Irian Jaya). In this report, we briefly review the present situation of these three human taeniid species mainly in Bali and North Sumatra. For community based epidemiological survey, we have adopted and applied questionnaire, microscopic observation of eggs, coproantigen tests, coproDNA tests, mitochondrial DNA analysis and morphology for isolated specimens, and serology for cysticercosis and taeniasis. For detection of taeniasis cases of both *T. saginata* and *T. asiatica*, questionnaire by expert doctors is perfect to detect worm carriers. Coproantigen test developed for detection of taeniasis of *T. solium* is also highly reliable to detect taeniasis carriers in the community. Epidemiological data from these two islands 2002-2005 are reviewed with backgrounds of historical culture, religions and customs.

## TAENIASIS/CYSTICERCOSIS IN PAPUA (IRIAN JAYA), INDONESIA

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Reports showed that an important parasitic zoonotic disease caused by *Taenia solium*, *Taenia saginata* and *Taenia asiatica* is found endemic in several areas of Indonesia e.g. in Papua, Bali and North Sumatra. At present, it is known that the highest prevalence of taeniasis/cysticercosis in Indonesia, caused by *T. solium* is among communities in Papua. In the early 1970<sup>th</sup>, 8-9% of stool samples from the Enarotali hospital, Paniai District was found positive with *Taenia* eggs. The samples were from members of the Ekari (Kapauku) ethnic group. Stool samples from the Moni ethnic group, living east of surrounding lakes, were egg negative. *Cysticerci T. solium* were discovered in pigs. During the years 1973-1976, cases of burns increased and were found to be connected with suspected cysticercosis cases. Among 257 cases of burns, 88 cases (62.8%) were suffering from epileptic seizures before or during hospitalization. In the year 1981, seropositive samples was mostly found in the endemic Obano village (16%). In 1997 the disease was discovered in Jayawijaya District, where is located east of Paniai District. Epilepsy, possibly caused by neurocysticercosis was detected in 53 patients among 155 cases. During 1991-1995, a local health unit in Assologaima reported 1,120 new cases with burns and 293 new cases of epileptic seizures among 15,939 inhabitants. Histopathologic picture and DNA were analyzed and found to be similar to those of *T. solium* from other regions of the world. Sensitive and specific serological diagnostic methods were used and improved. *Cysticerci* were detected in dogs, as well as in pigs. Copro-antigen test for detection of adult worms was carried out. Considering humans effective medical treatment with praziquantel for taeniasis and albendazole for cysticercosis, especially subcutaneous cysticercosis, which should be added with prednisone and sodium phenytoin in cases with neurocysticercosis, was conducted. Lifestyle, religion, and socio-economic aspects are important issues in the perpetuation and enhancing the endemicity of taeniasis and cysticercosis in Papua, Indonesia.

## **THE CUSTOM OF DANI'S PEOPLES: CONTEMPLATION OF MEDICAL ANTHROPOLOGY**

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This report describes how the serious health problems caused by cysticercosis and taeniasis in the Jayawijaya district can also be explained from the people's health behaviour. Methodology we used a qualitative approach and the methods used were depth interviews and observation for data collection. We interviewed head of tribe (kepala suku), men, and women. Research by the anthropologists in the Kurulu subdistrict in Jayawijaya district, Papua province (1993 and 2003) in Indonesia, show that from the social-cultural point of view, there are at least four main factors which put people at risk with the disease, due to the relation between cultural behavior (adat) and disease. These are: (1) the practice of traditional customs (adat); (2) dwelling patterns and interactions within the dwelling place ; (3) human to human contact; and (4) human to animal contact.

# **PEOPLE, PIGS AND PARASITES: RELATIONAL CONTEXTS AND EPIDEMIOLOGICAL POSSIBILITIES**

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Within Papua New Guinea, the relationship people have with their pigs varies between societies. These differences arise in the earliest phase of rearing piglets and result in domestic animals whose primary attachments are to other pigs, to places or to people. For Papua New Guineans, different pig management regimes fulfill ecological and social needs.

In addition, however, the ways in which pigs are raised and managed, and the presence or absence of a local population of wild pigs, have consequences for the exposure of both domestic pigs and people to parasites that they may host. Effective control of disease-inducing parasites should be attentive to society-specific relationships between people and their pigs.

## VACCINATION AGAINST CYSTICERCOSIS AND ECHINOCOCCOSIS

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Cysticercosis and echinococcosis are zoonotic infections caused by the larval stages of taeniid cestode parasites. One potential option to control these diseases in humans is to reduce transmission of the parasites in their respective animal host species, thereby preventing transmission to humans. Taeniid cestodes are unusual helminths in that immunity in the intermediate host plays an important role in the natural regulation of the parasites' transmission. In addition, a high level of immunity can be induced by vaccination with crude parasite extracts, particularly from the infective stage (oncosphere) contained within the parasite egg. Oncosphere antigens of a number of cestode species were analyzed in detail to identify particular proteins having the capacity to induce protection in vaccinated animals. These proteins were subsequently cloned as cDNAs and expressed in *E. coli* using pGEX vectors. The GST fusion proteins have been found to induce a very high level of protection against infection. Successful recombinant antigen vaccines have been developed in this way for *Taenia ovis* in sheep, *Taenia saginata* in cattle, *Taenia solium* in pigs and *Echinococcus granulosus* in sheep and other animals. Vaccine-induced immunity is associated with antibody and complement-mediated killing of the invasive oncosphere. While other immune mechanisms may also be involved in vivo, the protective efficacy of antibody alone is sufficient to account for the levels of protection induced by the vaccines. All of the protective oncosphere proteins identified to date have common characteristics, most particularly the inclusion of one or two modules identified as fibronectin type III (FnIII) motifs. Linear peptide epitopes have been characterized and the location of the immunodominant epitopes has been shown to be in a similar position for each antigen with respect to the proposed FnIII structure. However, available evidence indicates that none of these epitopes is host-protective and that protection is uniquely associated with conformational epitopes. The *E. granulosus* vaccine (EG95) is undergoing commercial scale-up and field trials in China. The more recently developed vaccine against *T. solium* infection in pigs (TSOL18/TSOL45) is undergoing optimization prior to field trials in Peru, Mexico and Cameroon. *T. solium* cysticercosis has been identified as an eradicable disease and this new vaccine has the potential to play an important role in the control or elimination of human cysticercosis.

## ***ECHINOCOCCUS GRANULOSUS*: VACCINATION OF DOGS WITH RECOMBINANT PROTEINS EXPRESSED SPECIFICALLY BY MATURE ADULT WORMS RESULTS IN SIGNIFICANT INHIBITION OF EGG PRODUCTION**

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Despite the substantial efforts made to control echinococcosis there is a clear need for new advances in its prevention. Dogs are pivotal in *Echinococcus granulosus* transmission and we contend that interruption of the parasite life cycle in the definitive host provides a very practical and cost-effective vaccination strategy to complement the recently developed Eg95 vaccine that targets infection in the intermediate host. To determine whether protection of dogs against infection was feasible, we immunized dogs three times subcutaneously with soluble proteins isolated from protoscolices (PSC) of *E. granulosus* and then challenged them with PSC (7 weeks after the first immunisation). In two independent trials, this resulted, at autopsy 72 (trial I) and 45 days (trial II) post-challenge, in significant repression of worm growth and suppression of egg production compared with parasites from adjuvant control dogs. We reasoned that the targets for immune attack in the vaccinated dogs are the products of genes associated with worm development and egg production. Accordingly, we used differential display PCR (DDRT-PCR) to screen expressed genes in two populations of adult worms: immature adult worms (IAW) collected from dogs at 35 days post-infection (*p.i.*) (at commencement of egg production) and mature adult worms (MAW) at 62 days *p.i.* (fully embryonated eggs present in the uterus). As the two worm types had similar strobilar development, we reasoned that the MAW contain newly transcribed mRNAs associated with the eggs themselves, as the MAW contain large numbers of eggs, or with egg development including the mature reproductive system. We subcloned the three genes of the family into an expression vector. The proteins expressed in *E. coli* were partially soluble in 30 °C. The soluble Glutathione *S*-Transferase (GST) fusion proteins were purified and vaccinated to dogs (3 × 80 µg/dog). The dogs were challenged and autopsied 45 days post infection (*p.i.*). The results showed that no worms developed to egg stage in the group vaccinated with EgM123-GST fusion protein. The recombinant proteins induced a significantly reduce of worms developing to egg-stage and worm burdens compared to the GST control group. RNA *in situ* hybridization showed that one of the gene (*EgM123*) family expressed mainly in testes of adult worm, indicating that worm test may be the target for the immune attacking.



## WHERE ARE THE TAPEWORMS ?

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My research group has been engaged for a long time in evaluating alternatives for controlling cysticercosis. Recently we participated with Marshall Lightowers in evaluating recombinant vaccines. For challenge of pigs we had to obtain fresh *Taenia solium* eggs, therefore one field trip to a rural community in Yucatan, with risk factors associated to cysticercosis, was organized by Rosanna Rodriguez-Canul in November 2003, 450 fecal samples were analyzed for coproantigen ELISA. Beginning January, cestocidal treatments were administered to 21 positive people followed by Epsom salt, no tapeworms were recovered. In a second trip in April 2004 to 2 rural communities in Oaxaca with similar conditions, we analyzed 200 fecal samples by ELISA, cestocidal treatments were administered the next day to 20 positive people followed by magnesium milk. Again, no tapeworms were recovered. To search for explanations to our failure we analyzed community based data published by various authors in the last 15 years, and found that: 1) Prevalence by coproantigen ELISA or by coproparasitoscopic studies in Mexico is 1.2% while in Peru is 2.5% and in Guatemala is 2.7%. 2) In Mexico tapeworms were recovered from around 37% of carriers detected by ELISA and from 66% of those that indicated having released proglottids after showing them pictures of tapeworms or fixed parasites; this latter approach increased in one year notification of taeniosis 6 times in an endemic jurisdiction of over 700,000 inhabitants. 3) In Peru the evaluation of purgatives in parasitological confirmed cases from hospitals and communities showed that gravid proglottids were expelled in 34% and 41% patients with castor oil and in 53% and 69% with polyethylene glycol salt, respectively. Therefore to ascertain where the tapeworms are, several possibilities should be analyzed: 1) Are tapeworms present in such low incidence in Mexico that carriers can be identified only by mass treatment of communities over 2000 inhabitants? 2) Is ELISA unreliable in spite of the fact that it is based in modern technology and has been used in several field studies? 3) Do tapeworms live in their hosts for short periods and are released spontaneously? 4) Are the purgatives commonly used not highly efficient, and thus tapeworms are not released? 5) Are there biological factors of tapeworms which are still unknown, therefore experimental models should be further evaluated? 6) Is *T. solium* taeniosis controlled in Mexico? If so, why are there still new human cases and young pigs with cysticercosis ?

## CONTROL PROGRAM FOR ECHINOCOCCOSIS IN SICHUAN, CHINA.

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Human echinococcosis (both *E. granulosus* and *E. multilocularis*) is a major health hazard in Western Sichuan. Ultrasound scanning has revealed that 12% of some populations are infected. Several factors contribute to the life cycles: For *E. multilocularis*, overgrazed pastures are suitable for rodents to multiply, and rodents are food for dogs. Most households keep guard dogs. There are also large numbers of unwanted dogs. For *E. granulosus*, sheep and goats have fertile cysts but yaks do not. Many sheep and goats die of old age (12-14 years) and are eaten by dogs, and their cysts are very infective for dogs. A New Zealand/China pilot hydatid control program initially tried to involve the Tibetan population in participatory planning, but was not successful because of the lack of mass media, the harsh environment, the predominance of illiteracy and the lack of knowledge about what caused hydatid disease. Very few children attended primary school. A program was set up and individual components were monitored, emphasising educating people about the hydatid life-cycles, and beginning some control procedures. After 4 years of education, only 43% had some knowledge and less than 30% made an effort to control the disease. Praziquantel pills were distributed to all households with dogs in the Spring and the Autumn, but a survey revealed that one third of all households were not giving the pills to the dogs. This was also revealed in results from coproantigen analysis of dog faeces. Where vaccination of sheep and goats to prevent echinococcosis was offered free of charge, up to 50% of households declined to participate. This was also revealed by analysis of random serum samples from sheep and goat flocks. Treatment of diagnosed human infections was inadequate. The people mistrusted surgery and the cost of travelling to hospital was prohibitive for many families even when free surgery was offered. Free albendazole therapy for up to 1 year was accepted but was palliative rather than curative. Better methods are needed to deliver the hydatid message to the individual households. Hydatid education could be linked to better health status through better hygiene. The people could devise their own hydatid control program based on the available options. Local government could facilitate participatory planning and coordinate control procedures.

# MATHEMATICAL MODELS FOR THE CONTROL OF CYSTIC ECHINOCOCCOSIS

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Cystic echinococcosis (CE) caused by *Echinococcus granulosus* is a global public health problem. In many areas the disease is being diagnosed in increasing numbers, whilst in other areas it is re-emerging due to the collapse of public health programmes associated with socio-economic changes. Mathematical models of the transmission dynamics between animals can have an important role to play in developing control options. In particular the parasite is highly endemic in many lower income countries where resources to undertake an intensive control programme that has been successful in wealthy countries such as New Zealand are not available. Data from dogs and livestock has been collected and modelled from a number of different countries and regions. In Australia and New Zealand, transmission modelling was first developed and these models have been refined using data from the Middle East and central Asia. The model indicates that relatively intense anthelmintic treatment of the dog population will result in a substantial decrease in the parasite population over time and has been supported by the results of control programmes. However, if the newly developed sheep vaccine is included in the control programme, then it should be possible to treat dogs less frequently to achieve the same result. This is due to the potentiating effects of attacking the parasite at two places in its life cycle. This should result in considerable cost savings over the use intensive anthelmintic treatment as the sole method of control.

## CONTROL OF CYSTICERCOSIS

Robert H. Gilman and the Cysticercosis Working Group in Peru

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Control of cysticercosis has been achieved in developed countries thru good sanitation and husbandry techniques. These advances are not expected to occur in most developing countries for years. Yet, in developing countries the chances for control programs to succeed have increased considerably due to a better understanding of the epidemiology of cysticercosis and the development of new, highly efficacious porcine vaccine candidates.

Mass treatment programs alone will not suffice since the biologic potential of tapeworm carriers is great. Also, a decrease in the porcine herd immunity to *Taenia solium* may occur thus permitting increased severity of *T. solium* infection in pigs and a subsequent increase in transmission.

New opportunities for control are based on evidence of both human and porcine clustering of infection. Clustering will make it possible to use new surveillance and treatment options that are targeted and thus more economical. Clustering may also permit ring vaccination techniques for pigs in close contact with tapeworm carriers.

Other techniques for controls will include compensation for cysticercotic pigs that come to slaughter followed by safe rendering so as to decrease clandestine slaughtering of pigs. Giving economic incentives for maintaining pigs in corrals and the development of safe community piggeries will be used in order to decrease the practice of permitting pigs to free range where they have ready access to feces.

All the above techniques will need international and national support. The decrease in both human and porcine cysticercosis however will provide dividends well beyond the cost of such a program.

## **PRESENT SITUATION OF TAENIASES AND CYSTICERCOSIS IN THE REPUBLIC OF KOREA**

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In the Republic of Korea, 3 species of human-infecting *Taenia* have been known to exist; *T. solium* (very rare), *T. saginata* (uncommon), and *T. asiatica* (relatively common). The national prevalence of *Taenia* spp. has been estimated every 5 years since 1971 through mass surveys on randomly selected inhabitants. The prevalence has been shown to decrease very rapidly; 1.9% in 1971, 0.7% in 1976, 1.1% in 1981, 0.3% in 1986, 0.06% in 1992, and 0.02% in 1997. In a most recent survey in 2004, no *Taenia* egg positive cases were detected among 20,546 randomly sampled inhabitants (about 1/2,239 of the whole population of South Korea). The same result of 0% egg prevalence was again observed in 2004 in another mass fecal examination on 4,176 people who visited health clinics. In 2001, however, out of 4,137 villagers on 45 southern and western coastal islands, 3 (0.07%) were found *Taenia* egg positive; 2 from Chuja-do (Island), Jeju Province and 1 from Hacho-do (Island), Jeonnam Province. Data on human cysticercosis are not enough to estimate its current situation in Korea. Based on a data (1968-1987) from imaging and serological tests, a total of 425 cases were diagnosed in Seoul National University Hospital. More recently, from 1996 to 2005, 523 (11.6%) of 4,495 sera examined in our Department were serologically positive for cysticercosis. Most (350 cases) of the seropositive cases showed the ELISA absorbance between 0.25 and 0.5 (cut-off value: 0.25), and 172 cases showed absorbance of 0.5-0.75, and 36 cases higher than 0.75. A proper interpretation is required on the rapid decrease of the egg prevalence of taeniasis v/s sustained seropositive rate of cysticercosis in the Republic of Korea.

## TAENIASIS ASIATICA IN THE PHILIPPINES

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Human infections with the adult tapeworms of *Taenia solium* and *Taenia saginata* have been reported in the Philippines as early 1907. While no systematic survey has been done to establish infection rate with either of the two tapeworms, taeniasis saginata was found more often than taeniasis solium. This data was based on mainly from the morphology of gravid segments submitted for identification at the diagnostic laboratory of the Department of Parasitology, College of Public Health. Meat inspection, on the other hand, reported pork cysticercosis. In the Philippines, it is assumed that transmission of *T. saginata* occurs through the consumption of infected raw or inadequately cooked beef as observed in many other countries. In this report, *Taenia* segments identified as *T. saginata* from six Filipino patients were examined for mitochondrial DNA through the courtesy Dr. Akira Ito and Dr. Hiroshi Yamasaki. Results showed that five of the six specimens studied are *T. asiatica* and not *T. saginata*.

## PRESENT SITUATION OF TAENIASIS / CYSTICERCOSIS IN LAOS

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*Taenia* spp. infection and cysticercosis are recognized to occur in Laos but there were not much data published. According to different surveys carried out by the Institute of Malariology, Parasitology and Entomology in different areas of the country from 1990-1994, the prevalence of *Taenia* spp. infections was 8.19%. Results reported by some investigators were as follows: 3.0% in three villages of Khammouane Province (Vannachone *et al*, 1996); 0.67% in seven districts of Vientiane Province (Phetsouvanh *et al*, 1999). Parasitological surveys among primary schoolchildren were carried out on a national scale from May 2000 to June 2002, and the prevalence of *Taenia* spp. infections was 0.6% (Rim *et al*, 2003). Stools examination done in Mahosot hospital, the biggest in Laos, revealed the number of 0.74% and 0.61% in 2003 and 2004 respectively. These prevalence rates seem not to be a health problem for Lao people, but these numbers were probably underestimated. Lao people have behavior in eating raw meat. Results of the interviews in Vientiane Province demonstrated that about 75-77% have accepted having eaten either raw or undercooked meat from pork or beef from sometimes to every week (Phetsouvanh *et al*, 1999). In Saravane province, 88.8% and 46.9% of villagers were used to consuming raw or undercooked beef and pork respectively, and *Taenia* spp. infection was 5.0% (Sayasone *et al*, 2004). In rural areas of Laos, cattle are bred in the field and pigs are laid freely running around the village and feed waste under the house including human waste so they can be easily infected with *Taenia*'s eggs and a rather high prevalence of human infection of taeniasis should be expected. Neurocysticercosis were diagnosed occasionally because CT Scans were not available in most of hospitals. Serological tests for cysticercosis were not used in any laboratory. These sophisticated techniques are not adopted by the least developed country of the region like Laos. Epilepsy cases were found in different regions of the country but the relation with cysticercosis was not confirmed. A study on Epilepsy was conducted in Hinheup District of Vientiane Province in 2003 and 2004 by investigators from the French-speaking Institute of Tropical Medicine in Vientiane. Thirty sera from epilepsy cases and 120 sera from controls were analyzed in laboratory of Asahikawa Medical College using ELISA and immunoblot techniques. The result showed that four sera were positive for cysticercosis by ELISA and two sera were positive by Immunoblot. Practically, species of *Taenia* were not identified, but it seems that most of cases were *Taenia saginata*. Cysticerci were frequently found in beef, but there were rare in pork. *Taenia asiatica* is probably existed in Laos because this new species of *Taenia* were reported in neighboring countries like Thailand and Vietnam.

In conclusion, taeniasis and cysticercosis are prevalent in Laos, but there were only a few surveys on this parasitic disease. Further studies in large scale are recommended by using serological tests and molecular biology techniques.

## TAENIASIS/CYSTICERCOSIS AND ECHINOCOCCOSIS IN THAILAND

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Taeniasis is one of the major food-borne parasitic zoonoses in Thailand. During the years 1957-1997, the prevalence was low in most parts of the country, except for the South. Recent (2000-2005) country prevalence was lower than 1%. A high prevalence (5.9%) was found among 1,450 villagers from 30 villages in the North, and among 1,233 stool samples from 19 provinces in the Northeast (2.8%). *Taenia saginata* was the dominant species. Cysticercosis in Thailand is somewhat under-reported/recorded. During the period 1965-2005, diagnosis was based on techniques other than serodiagnosis, giving a total of cysticercosis cases of less than 500. However, an immunoblot technique using delipidized cyst antigen showed 314 positive cases out of 754 samples tested in 2000-2005. Reports of neurocysticercosis appeared more often than cutaneous cysticercosis. A total of 24 cases of echinococcosis, mostly hydatid cysts (only 2 cases of alveolar cysts), were recorded from 1936-2005. These records included 3 cases of foreigners seeking surgery in hospitals in Bangkok. Most Thai patients were migrant workers from the Middle East, and only a few cases were indigenous. The prevalence of cysticercosis and echinococcosis is increasing resulting from sensitive modern diagnostic tests. Taeniasis will persist in Thailand as the consumption of raw/half-cooked meat dishes is still a normal practice for Thai people.



## PRESENT SITUATION OF TAENIASIS/CYSTICERCOSIS IN VIETNAM

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Taeniasis/cysticercosis has been recognized and reported in Vietnam since the early 20<sup>th</sup> century, however, available epidemiological data were sparse and only from community-based studies in a few provinces in Vietnam. Slaughterhouse- and hospital-based data and small population-based studies form the bases of this report. Abattoir records indicated that porcine and bovine cysticercosis were present in several provinces from the north to the south of the country. The prevalence was low, probably due to an underestimation since several infected pigs had not been sent to public slaughterhouses. Occasional inspection of 737,460 pigs and 144,390 cattle at markets in Hanoi showed the meat infection of 0.05 % and 0.03%, respectively. Human cysticercosis and taeniasis have been found in most of the provinces in Vietnam. Rates of taeniasis, as determined by stool examination for ova, have also been reported from 0.1 and 6% in the community in the northern and central of Vietnam, but without distinction between *Taenia solium* and *Taenia saginata*. In Hochiminh city, about 30 to 50 persons come to clinics every year due to proglottid detection, and all these proglottids were morphologically identified as *T. saginata*. Available serological and neuro-imaging techniques at referring hospitals have increased the chance of detection. In HoChiMinh city, about 100 to 150 cases were serologically diagnosed at hospitals. A preliminary survey in the north showed a seroprevalence of cysticercosis between 2% and 5%. In addition to *T. solium* and *T. saginata*, *Taenia asiatica* was recently detected in Vietnam. *Cysticercus cellulosae* was also found in dogs at 0.14% in the north. In 1998, one case was reported at Dong Nai in the south. In some areas of the country, traditional pig husbandry systems, inadequate meat inspection, poor sanitation, and particularly the behaviors of eating unclean fresh vegetables, and using human feces as fertilizers may facilitate the transmission of *T. solium* and *T. saginata*. The current findings suggest a widespread presence of human tapeworm carriers, and thus a high risk of human cysticercosis in both rural and urban settings in Vietnam. Infected persons were of various local settings, age groups, and occupations. This is due to a small scope of the studies that a general and representative figure could not be drawn. More surveys with representative and adequate sample size are evidently needed.

## CYSTICERCOSIS/TAENIASIS SITUATION IN NEPAL

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Postmortem surveys study of pigs at slaughter establishments in Kathmandu, Sunsari, Syangja and Tanahun districts was carried out for cysticercosis; to have antemortem detection of *Taenia solium* infection of pigs in a community by lingual examination; to survey human stool sample test for the prevalence of taeniasis eggs in the ethnic groups like Gurung, Magars, Sarkies, Darai and Bote; and to review human cysticercosis cases on the basis of hospital-based data. In Nepal this was the first time a proper and thorough risk assessment of the situation involving both the human health and pig farmers was carried out in order to determine whether and how the *T. solium* in pigs situation in Nepal should be managed. Survey was carried out and similarly the secondary data were collected and presented on the basis of the report of the human and porcine cysticercosis study in different parts of Nepal. Direct microscopic examinations of human stool samples were also carried out. The overall intestinal helminth prevalence rate in human was found to be 60%, while the taeniasis prevalence rate was 18%. Comparatively Magar ethnic groups occupationally do pig farming and frequently consume pork rather than other ethnic groups. Out of the two hundred and fifty pigs examined, thirty-four (14%) were found positive for porcine cysticercosis. Twenty-two (15%) positive samples were from Khichapokhari, six (12%) samples were from Koteshwor KMC and six (11%) were from Dharan. During the study period a total of 419 pigs were examined for the lingual visualization and palpation for the cyst in Syangja, Tanahun and Kathmandu districts. Out of total samples studied, 32% of them were found positive. Cysticercosis infection in between male and female were statistically analyzed calculating *p* value which showed significance difference between sex ( $p=0.003$ ). The collected data on both taeniasis, human and porcine cysticercosis indicated that the disease is very severe and developing as a serious public health importance.

## ***TAENIA SOLIUM* CYSTICERCOSIS IN INNER MONGOLIA, CHINA**

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For detection of human *Taenia solium* cysticercosis, enzyme-linked immunosorbent assay (ELISA) and immunoblot (IB) methods using glycoproteins purified by preparative isoelectric focusing were applied for local residents in Tongliao area, Inner Mongolia, China in 1998. Approximately 89% (39 of 44 patients suspected to be neurocysticercosis (NCC) at Tongliao City Hospital) in- and out-patients were residents of Inner Mongolia. About 53% were male patients and 77% belonged to working age group ranging from 18 to 59 years old. Agricultural population was 32%. Of 44 patients, 31 cerebral computed tomography (CT) scan positive patients were serologically confirmed to be cysticercosis. Other patients without any lesions in CT scan negative patients showed lower OD values similar to those of normal serum. These findings confirm that both ELISA and IB assays have a high correlation, and are sensitive to detect cysticercosis patients, either asymptomatic or symptomatic.

## TAENIASIS/CYSTICERCOSIS IN EASTERN QINGHAI PROVINCE, CHINA

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Objective: To know the epidemiological situation of taeniasis/cysticercosis in human and pigs in eastern Qinghai province. Methods: (1) Human's examination including: Asked the history of draining taeniid segments; Detected taeniid eggs using scotoscopy in faeces; Examined antibody on taeniasis/cysticercosis with ELISA; The results were checked by CT/MRI. (2) Pig's examination: examined cysticercosis of the slaughtered pigs. Results: (1) From 1997 to 1998, a field survey of epidemiology on taeniasis/cysticercosis was carried out at Huzhu county in eastern Qinghai province. The results show that the infection rate of human taeniasis was 0.39% (4/1,024), the sera positive rate of human cysticercosis examined with ELISA was 14.71% (159/1,081), the incidence was 0.56% (6/1,081); The pig's infection rate with cysticercosis examined with ELISA was 7.23% (17/235) and the incidence was 4.60% (11/239). (2) From 2001-2002, a field survey of the epidemiological situation on taeniasis/cysticercosis was carried out at different areas in eastern Qinghai province including Minhe, Ledu, Pingan counties and Xining city. The results showed that infection rates of taeniasis were 0.43% (2/470), 0.10% (2/2,025), 0.15% (3/1,952) and 0% (0/1,960), respectively, and the serum positive rates of cysticercosis with ELISA in crown were 5.88% (28/476), 3.53% (15/425), 0.29% (1/347) and 0.26% (1/384). (3) From 1990 to 2004, we had cured 108 patients with neurocysticercosis including 65 male-cases and 43 female-cases. Conclusion: The taeniasis/cysticercosis in human and pig are serious in eastern Qinghai province. The main factors that cause widespread distribution of taeniasis/cysticercosis at native areas are: The patients who infected taeniasis, existing in a long period of time; The mass lack of the knowledge of the prevention and treatment on this disease; A part of families has not toilet or their toilet are opened; Excrement which has not been harmless was applied fertilizer directly; The personal hygiene condition is bad; Large quantity pigs are being bred in native families; Peasants have some bad habits as they slaughter pig infected with cysticercosis and eat this kind of meal, even they buy this kind of pig meal which infected with cysticercosis to eat or sell it to public.

## TAENIASIS/CYSTICERCOSIS INFECTIONS IN GUANGXI ZHUANG AUTONOMOUS REGION, SOUTH CHINA

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Guangxi Zhuang Autonomous Region, located in south China, is high endemic area of taeniasis/cysticercosis infections, but the studies on the disease in the region are very limited. Few basic sero-epidemiological studies and clinical case analysis have revealed that 41 of 63 counties are endemic with taeniasis/cysticercosis in the region. Recorded *Taenia* species include *Taenia solium*, *Taenia saginata* and *Taenia asiatica* are still debated. The highest infectious rate of *T. saginata* was reported as 65.93% in Miao ethnic population. Highly endemic areas in north and west parts of the region is likely attributed to local diet habit, poor sanitation condition and style of livestock breeding. The distributions of the *Taenia* species and other epidemiology information of *Taenia* in most counties, especially in places where ethnic groups live, are still not available.

## **PRESENT SITUATION OF TAENIASIS/CYSTICERCOSIS IN MALAYSIA**

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## **PRESENT SITUATION OF TAENIASIS AND CYSTICERCOSIS IN PAPUA NEW GIUNEA**

Ifor L. Owen

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There is no evidence that taeniasis due to *Taenia solium* is present in Papua New Guinea (PNG), but there is serological evidence that human cysticercosis exists at particular locations near the border with West Papua (Indonesia), where refugees from across the border have been settled. Only a few surveys have been conducted; the first was in 1986, when one refugee who originated from an infected locality in West Papua was found to be serologically positive, using Western blot. Subsequently, there have been unpublished reports of more positive cases amongst refugees and, it is claimed, amongst local inhabitants that live near the border. A serological survey conducted in PNG at the southern end of the border with West Papua revealed no positive cases of cysticercosis. There are no reports of pigs or dogs affected with cysticercosis in PNG.

## **FUTURE CONTRIBUTION OF FAP**

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More than 40% of the world's population live in Asia, and Asian countries share common geographic and social environments. Asia is an important endemic region of parasitic diseases in the world. Similar species of parasites and parasitic diseases are found throughout Asia. On the other hand, there are unique features of parasitic diseases according to the endemic or epidemic regions, because there are extreme heterogeneity and polymorphism in races, histories, cultures, religions, economics, political systems and other factors. Parasitic diseases should be conquered by understanding life styles of people and their circumstances. We should pay respect their/ our own cultures and life styles that have been succeeded from ancestors, and we should be proud of them. Therefore, it is of importance to establish the strategy for Asian people being suffered from parasitic diseases by Asian parasitologists ourselves who live together with patients with parasitic diseases. Asian countries have been interchanged and affected geographically and historically. Thus, we can share the problems of parasitic diseases that link to the life styles and geographic and cultural circumstances, and we are encouraged to collaborate with each other to overcome the problems. In order to overcome the parasitic problems beyond the boundaries of Asian countries, we take measures to cope with a new movement, cooperation between Asian parasitologists by establishing a union of Asian parasitologists. Based upon the idea of "Asia is one (Tensin Kakuzo Okakura)"(1), we founded Federation of Asian Parasitologists (FAP) on November 13, 2000 by holding 1<sup>st</sup> Congress of FAP at Chiba, Japan. FAP aims to address the problems of parasitic diseases in Asian countries, and to promote parasitology sciences in Asia, through cooperative scientific strategies. FAP encourages parasitologists to present the most up-to-date tools and knowledge for control of parasitic diseases, and FAP will also offer ways to use already established scientific tools to make them more effective. These major aims of FAP are in keeping with the traditions of Asian parasitology that were established by our seniors. We are going to hold 3<sup>rd</sup> Congress of FAP that is convened by Prof. Akira Ito in conjunction of "Taeniasis/Cysticercosis and Echinococcosis International Symposium with Focus on Asia and the Pacific" on July 5-8, 2005. Expanding of membership number by inviting parasitologists from uninformed Asian countries and activating our volunteer contribution to people being suffered from parasitic diseases by holding 4<sup>th</sup> Congress of FAP, establishing the network between EMOP, EPS, FLAP, NAPS, WFP, and WHO, publishing the second version of Asian Parasitology, and establishment of economic autonomic system for FAP volunteer activities.



## **SOUTHEAST ASIAN TROPICAL MEDICINE AND PARASITOLOGY NETWORK**

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The SEAMEO TROPMED Network is a regional cooperative network established in 1967 for education, training and research in tropical medicine and public health under the Southeast Asia Ministers of Education Organization. The Network operates through four Regional Centres with respective areas of specialization and host institutions: Community Nutrition/Tropmed Indonesia; Microbiology, Parasitology and Entomology/Tropmed Malaysia; Public Health/Tropmed Philippines; and Tropical Medicine/Tropmed Thailand. To train health workers, to support research on endemic and newly emerging diseases, and to advocate relevant health policies are the main functions of these centres.

SEAMEO TROPMED Network in collaboration with the Faculty of Tropical Medicine, Mahidol University and other institutions has regularly organized the Seminar on Food-borne Parasitic Zoonoses every 3-5 years over the past 15 years. The Faculty of Tropical Medicine has organized the annual Joint International Tropical Medicine Meeting since 1996. Full papers of the presentations at these two meetings have been published as supplementary issues to the Southeast Asian Journal of Tropical Medicine and Public Health, an in-house journal of SEAMEO TROPMED Network. Recently, the Parasitology Association of ASEAN Countries has rotated the hosting of the Asean Congress of Parasitology and Tropical Medicine. These institutional and conference networks will enable closer links, to promote the health of people in the Southeast Asian region.

## **INTERNATIONAL ELECTRONIC RESOURCES IN VETERINARY PUBLIC HEALTH**

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Veterinary public health with special emphasis on zoonoses and food safety has emerged as a leading area of activity in the post WTO scenario. Under the present circumstances it becomes imperative for workers to keep themselves abreast with the global developments in this area. Electronic resources available on the World Wide Web are quite helpful in this respect. Most of the information is freely accessible on the Internet where simple registration can provide regular updated information on your desk. There are several resources under the aegis of FAO/WHO/OIE and other agencies, which can be accessed to get information. The websites of FAO/WHO/OIE have special pages dealing with veterinary public health and food safety concerns. Several regional networks have been established to update the information in the respective regions by collecting information from the Internet. One such network is functioning for the Asian countries by the name of “A Network of Veterinary Public Health and Zoonotic Diseases in Asia”. This network can be accessed at <http://www.vphasia.org>. This network started in 2003 is primarily catering to the workers in Asian countries and building and disseminating information to members. The primary objective of the network in addition to post information to members is to help forge linkages between individuals and institutions to develop strategies for the control of diseases of veterinary public health concern.

## **VETERINARY PUBLIC HEALTH ACTIVITIES AT FAO: CYSTICERCOSIS AND ECHINOCOCCOSIS**

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In many developing and transition countries, parasitic zoonoses such as cysticercosis and echinococcosis, cause serious human suffering and considerable losses in agricultural and human productivity, thus posing a significant hindrance to their development. Although, effective and reliable tools for the diagnosis, prevention and control of parasitic zoonoses are now available, their implementation has not always been successful in many countries. This is primarily due to the lack of awareness on the presence or impact of the causing parasites (*Taenia saginata*, *Taenia solium* and *Echinococcus* spp.). In addition, often the needed intersectorial cooperation, resource management and political commitment for their control are (also) absent. FAO's regular programme has established a global network of professionals directly involved in zoonotic and food borne diseases. The network provides a basic framework for the spread of information related to the diagnosis, prevention and control of major zoonotic diseases including cysticercosis and echinococcosis.

## **TOWARD CONTROL OF TAENIASIS/CYSTICERCOSIS AND ECHINOCOCCOSIS IN CHINA**

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From 2001 to 2004, a nation-wide survey on current situation of major human parasitic diseases was organized by the Ministry of Health in 31 provinces/autonomous regions/municipalities in China. The main results showed that the infection rate of soil-borne nematodes has declined remarkably. While the infection rate of food-borne parasitic diseases has been increased significantly in some localities. It is showed that the infection rates of soil-borne nematodes, taeniasis, and echinococcosis were all significantly higher in the western areas than the eastern and central areas. Based on above epidemiological characteristics, the following control strategies will be performed: (1) Formulating a medium- and long-term national control program for major parasitic diseases. (2) Putting emphasis on control of echinococcosis in western China. (3) Performing integrated control strategies with supports from different departments of the government.

## CHALLENGES IN CONTROLLING TAENIASIS/CYSTICERCOSIS IN INDONESIA

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In Indonesia three important *Taenia* species were identified namely *Taenia solium*, *Taenia saginata* and *Taenia asiatica*. Taeniasis/cysticercosis was reported from several provinces of Indonesia namely Papua, Bali, North Sumatra, Lampung, East Nusa Tenggara, West Kalimantan, North Sulawesi and South-east Sulawesi. The highest level of endemicity of taeniasis/cysticercosis was found in Papua (Irian Jaya). Recent surveys in 2000 and 2001 in Jayawijaya District, Papua showed that 5 (8.6%) of 58 local people harbored adult tapeworms, whereas 46 (47.9) of 96 local people, 50 (70.4%) of 71 pigs, and 7 (11%) of 64 local dogs were seropositive for cysticercosis. The magnitude of the problem of the disease in the country is not known. It is suspected that the disease is present in areas with widely distributed pig or cattle population together with poor sanitation. The ratio population of pig to human in 1999 was 1 over 29. However, human and porcine cysticercosis is expected to be infrequent in areas where the majority of the people are Moslems. Poor personal hygiene, eating habits and sanitary practices such as no latrines and inadequate pig and cattle farming has made control of the disease difficult. The disease is not a priority in health sector as well as in veterinary, both at the central and local government. However, limited efforts toward control of the disease have been conducted such as training to health personnel, education to community on the disease prevention, and providing anthelmintics. Working group for control of the disease in Indonesia and an international collaboration has been established between Ministry of Health, Indonesia, University of Indonesia, and Asahikawa Medical College, Japan since 1996-1997. For the future, strengthening of laboratory capacity, advocacy to the districts, legislative measures consisting of meat inspection and cattle or pig husbandry are among factors that should be taken into account in developing the strategic plan to control the disease. Considering the differences among communities regarding socio-cultures, religions, education and socio-economic levels, the control programmes should be adapted to the local situation.

## **ESTABLISHMENT OF A NETWORK FOR CONTROL OF CESTODE ZOOSES IN ASIA AND THE PACIFIC**

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The 21st century has started with facing the emerging infectious diseases basically due to over populations in humans and animals with lack of sufficient foods or energy, political, religious and economic struggles resulted in increase in the number of refugees, immigrants, and global economic trading including agricultural products under relatively poor hygienic conditions due to relative poverty in developing countries not only in Asia and the Pacific but also in almost all over the world. It is easy for us to expect that there is no more crucial barrier between developing and developed countries, since every thing is moving so fast every where. One of the most serious public health issues under such situation is how to prevent and control emerging and reemerging infectious diseases including parasitic diseases. At this symposium, we are discussing on how to challenge for prevention, surveillance and control of cestode zoonoses based on peer and applied science and technology plus social or human science. It is the most important for us to develop reliable tools for detection of people or animals infected with these parasites. Such original contributions are expected to be shared with colleagues who are facing how to diagnose or do field survey etc. Alternative approach is political strategy how to control such diseases which have been challenged by WHO and FAO. Politicians can improve drastic changes in such public health issues. However, it is based on evidence-based-scientific data anytime and anywhere. Recently, to establish the network system is some kind of new fashion, which is rather expected to be carried out through WHO or FAO activities. Without original contribution in science or technology, it is impossible to evaluate the local data or share the science or technology with local colleagues. As mentioned at the preface, the sentence "Dig the very place where we stand on, then we will find a good spring." is the key word at least for me to do collaboration projects in foreign countries. We all facing how to prevent and control of such parasitic diseases have to develop our own strategy using our own materials and skills and improve these with scientific, technical and financial support from other groups. Such cooperation and collaboration are through reliable human relationships. As Asia and the Pacific is one of the highly attractive areas in research or politics or new type of business to get research fund by saying blah, blah, blah and buy the local data without scientific evaluation, we have to remind that we are not the invaders. We all have to remind ourselves what we are and we have to contribute based on our own original contribution other than money. In order to establish a network for communication of updated information on these cestode zoonoses, it is essential to do collaboration projects. Nothing can be done without local collaborators. Local data are by local people for local people. We are happy to join with such projects as collaborators or consultants and live together.

## **P1 PREVALENCE AND RISK FACTORS ASSOCIATED TO TRANSMISSION OF TAENIASIS/CYSTICERCOSIS IN SOUTHERN MEXICO**

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A comprehensive study on *Taenia solium* taeniasis/cysticercosis was performed from 1996 to 2005, in six rural communities from southern México. First, a census and a questionnaire designed to collect socio-economic data, sanitary facilities and general information about the life cycle of *T. solium* was performed in each community. Then, after informed consent, a blood and a faecal sample was collected from all willing individuals. And a blood sample was collected from pigs after their owner's consent. Prevalence and seroprevalence were estimated as the number of infected and/or exposed individuals from the total analysed. Risk factors were estimated by  $X^2$  using Odd ratio in Epi Info 6.0. Seroprevalence of human and porcine cysticercosis was assessed using an immunoblot specific to the region of 26 kDa. Prevalence of taeniasis was estimated by microscopy and by a coproantigen-ELISA (CoAg-ELISA). In the first community [Tedzidz (20°58'N, 90°12'W)], seroprevalence of human cysticercosis was of 3.7% (5/134), porcine cysticercosis of 35% (26/75). Prevalence of taeniasis was of 1.5% (7/475). In Texán de Palomeque (20°60'N, 90°19'W), Seroprevalence of human cysticercosis was of 3.8% (9/238)m, and porcine cysticercosis was of 5.6% (13/231). Prevalence of taeniasis was of 0.8% (12/1518). In Tixcacaltuyub (89°94'N, 20°52'W), seroprevalence of human cysticercosis was of 4% (6/150) and of porcine cysticercosis was of 26.5% (69/260). Prevalence of taeniasis was of 0.7% (6/866). In Kochol (20°37'N, 90°10'W), seroprevalence of human cysticercosis was of 3% (7/226) and of porcine cysticercosis was of 12% (47/400). Prevalence of taeniosis was of 1.7% (13/748). In Santo Domingo (20°39'N, 90°11'W), seroprevalence of human cysticercosis was of 2% (12/602) and of porcine cysticercosis was of 2.7% (2/73). Prevalence of taeniosis was of 2.0% (16/769). In San Rafael (20°36'N, 90° 9'W), seroprevalence of human cysticercosis was of 2.3% (8/350) and of porcine cysticercosis was of 0.5% (1/183). Prevalence of taeniosis was of 0.6% (3/471). In the case of human taeniasis and cysticercosis, there were no differences by gender and groups of age. In all cases, the main risk factor associated to transmission of taeniasis was eating infected pork meat and for cysticercosis living in close proximity to a *T. solium* carrier. For porcine cysticercosis, was confinement of animals, specially, free range pigs with easy access to the areas designated as toilets. Traditional pig husbandry practices, lack of adequate sanitary services and lack of knowledge about the life cycle of *T. solium* are key factors associated to transmission of *T. solium* in southern Mexico.

## **P2 CHEMOTHERAPY OF PORCINE CYSTICERCOSIS WITH ALBENDAZOLE SULPHOXIDE**

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The cestode *Taenia solium* is recognised as a significant threat to human health and livestock throughout the developing world. The life cycle includes pigs as a normal intermediate host harbouring the larval stage (metacestode), and humans as the definitive and accidental host for the adult intestinal and larval stage respectively. Chemotherapy of infected pigs with metacestodes (cysts) of *T. solium* is a possible strategy for avoiding disease transmission to humans. In this preliminary study: 7 naturally infected pigs (age range 6-12 months) were allotted to treated group (n=4) receiving a subcutaneous injection of Albendazole Sulphoxide (ABZSO) 15 mg/kg/body weight/day for 8 days. The control group (n=3) received NaCl 9%. After 12 weeks, all animals were slaughtered and at least 200 metacestodes were isolated from muscle and brain. Using histology and the metacestode viability criteria described in this study, treated pigs had no viable cysts in muscle (0/200) but 41.1% (7/17) of viability was observed in their brains. In the control group, 91.5% (183/200) and 75.8% (22/29) of viability was observed from muscle and brain respectively. Treatment of pigs with a 15 mg/kg dosage of subcutaneous ABSZO (Pisa, Mexico), applied daily for 8 days was 100% effective in the control of muscular cysticercosis. This dosage, was insufficient however, to affect the morphology and viability of cerebral cysticercosis. The macroscopical and histological appearance and the results of viability test provide evidence that the parasites are killed, but more time is needed for total disappearance of degenerated metacestodes from the meat.



### **P3 PREVALENCE OF SWINE CYSTICERCOSIS IN THREE RURAL COMMUNITIES FROM SOUTHERN MEXICO**

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Swine cysticercosis produced by the larval stage of *Taenia solium* is highly prevalent in Mexico. Its transmission has been associated to low sanitary facilities, poor hygiene and traditional methods of backyard farming; allowing pigs to roam free. In this study an immunoblot and the tongue palpation (TP) method were used to assess prevalence and to evaluate risk factors in three rural communities from July of 2002 to November of 2003. After informed consent, a blood was collected from the jugular vein of pigs. Prevalence and seroprevalence were estimated as the number of infected and/or exposed individuals from the total analysed. Risk factors were estimated by  $X^2$  using Odd ratio in Epi Info 6.0. A total of 231 pigs from 1-48 months old were recorded in the three communities; 198 free-range pigs, 18 tied pigs and 15 confined pigs. In Kochol (20°37'N, 90°10'W), seroprevalence of porcine cysticercosis was of 12% (47/400) and of 5.5% (22/400) by TP. In Santo Domingo (20°39'N, 90°11'W), seroprevalence by immunoblot and prevalence by TP was of 2.7% (2/73), respectively. In San Rafael (20°36'N, 90° 9'W), seroprevalence and prevalence by TP was of 0.5% (1/183), respectively. There were no differences by groups of age and no differences between boars and soars. The major prevalence was observed in the free range pigs with easy access to the areas designated as toilets. Traditional pig husbandry practices promote perpetuation of the life cycle of *T. solium* in rural communities.

#### **P4 TAENIA SOLIUM IN CHINCHILLA LANIGER : PROS AND CONS FROM AN EXPERIMENTAL MODEL OF GRAVID TAPEWORMS**

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*Chinchilla laniger* has been reported as an experimental definitive host for *Taenia solium*. In the present study we evaluated infection rate, tapeworm development and amount, viability and infectivity of eggs recovered. Six experiments were performed at different times over a 3 year period using in total 76 outbred female chinchillas and infecting with 4 to 6 cysticerci per host. Chinchillas were immunodepressed with 6 mg of methyl-prednisolone acetate every 14 days starting the day of infection. Prior to infection, viability of cysticerci by *in vitro* evagination was tested. Coproantigen ELISA or sieved faeces were used to follow infections. Infection rate of chinchillas was 30% (22/76), 38 tapeworms were recovered from the 76 chinchillas that were infected with 352 cysticerci, therefore efficiency of infection was 10%, from the 38 tapeworms recovered 14 were gravid (37%), each gravid proglottid had between 10 and 5,000 mature eggs. In one experiment of 2 piglets were infected and viability was tested with all methods published for this purpose. Viability was 7% by MTT reduction, 30% by oncosphere activation, 36% with neutral red, 54% by trypan blue and 60% with propidium iodide, indicating that, except for the last two methods, values are not comparable. Pigs were infected with 50,000 eggs each; after counting the number of cysticerci recovered at necropsy, performed 3 months after infection, 0.3 to 0.4% infection rate was obtained. Cysticerci with 100% evagination were used in 4 experiments, while in the other two, cysticerci had 70% and 84% evagination, no infected chinchillas were obtained in one and no gravid tapeworms were recovered in both experiments. Along the 5 months that infections have lasted coproantigens showed an irregular distribution, while proglottids in feces, detected since week 9 of infection, showed a periodic release. Our results indicate that the experimental model of *T. solium* taeniosis in *C. laniger* is a good alternative for providing eggs and adult tapeworms to be used in different types of experiments. Nevertheless more studies are needed to optimize this model.

## **P5 GENETIC POLYMORPHISM IN *TAENIA SOLIUM* CYSTICERCI RECOVERED FROM EXPERIMENTAL INFECTIONS**

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Random amplified polymorphic DNA technique (RAPD) showed that *Taenia solium* cysticerci recovered from naturally infected pigs from Mexico, Honduras and Tanzania have a clonal structure and local lineages with probable events of genetic recombination without genetic flow within them. To evaluate the genetic polymorphisms from cysticerci recovered from experimental infections in pigs, using *T. solium* eggs obtained from human carriers, four 2 month old- piglets from a certified farm were infected with eggs obtained from three *T. solium* adult worms: A: 10 year old female, B: 25 year old female, C: 44 year old male, the 4<sup>th</sup> pig was infected with a mixture of eggs from the three tapeworms. Each pig was inoculated *per os* with 50,000 eggs. After 16 weeks pigs were humanely euthanized and cysticerci were excised. Ten parasites recovered from each pig were analyzed by RAPD using commercial primers OPB11; 14 and 18. Proportion of polymorphic alleles, mean heterozygosity, Nei's genetic distances  $D$  and the UPGMA dendrogram were obtained with the TFPGA program. Percent establishment was: 2.2, 4.2, 0.2 and 0.6 (tapeworms A, B, C and the mixture, respectively); 36 loci were identified, 83% polymorphic, average heterozygosity was 0.016; the dendrogram clustered the cysticerci into two main clades: one included cysticerci from tapeworm A and some parasites from the mixture and from tapeworm B, while the other had most cysticerci from tapeworm B, the mixture and all from tapeworm C. Our data of polymorphic loci ( $P= 0.14-0.55$ ) and heterozygosity ( $H= 0.06-0.22$ ) were slightly higher than those published for natural infections ( $P= 0.18-0.40$  and  $H= 0.03-0.16$ ). The small number of cysticerci used may account for these results, alternatively, cysticerci recovered from experimental infections might have a higher polymorphic genetic pool than those coming from natural infections, because environmental and genetic selection forces present in nature, such as tolerance to desiccation and natural selection for some genotypes, influence natural infections but do not participate in experimental ones.

## **P6 DETECTION OF *TAENIA SAGINATA ASIATICA* COPROANTIGEN BY SANDWICH ELISA DURING THE EXPERIMENTAL INFECTION IN HUMANS**

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Coproantigen, egg and proglottid excretion of *Taenia saginata asiatica* were investigated during the course of human experimental infections. Excretion of proglottids and eggs were observed from 4 out of 5 volunteers after inoculating with the mature cysticerci recovered from either SCID mice or gerbils. Prepatent period of the infection ranged from 80 to 168 days. The diagnostic sensitivity of the egg examination and proglottid observation during the patent period were determined individually, and ranged from 58 to 100% and 44 to 95% in the 4 volunteers, respectively. Coproantigen of *T. s. asiatica* during the infection was evaluated by the sandwich enzyme-linked immunosorbent assay (ELISA) using polyclonal antibody to excretory and secretory (ES) antigen of the parasite. The assay showed no cross-reactivity with ES antigen of *T. solium*, and somatic antigens of *Diphillobothrium latum/nihonkaiense*, *Fasciola hepatica*, *Paragonimus westermani*, *Schistosoma japonicum* and *Entamoeba histolytica*. But the coproantigen ELISA showed cross-reaction with somatic antigen of *Ascaris suum*. The diagnostic sensitivity of coproantigen ELISA was determined individually, and ranged from 0 to 55% during the prepatent period and 88 to 100% during the patent period in the 4 volunteers. One out of the 4 volunteers showed no coproantigen ELISA positive during the prepatent period, giving a diagnostic sensitivity of 0%. However, the other 3 volunteers showed the diagnostic sensitivity obviously increased ranging from 45 to 100% in the latter half of the prepatent period, comparing with the diagnosis sensitivity ranged from 0 to 26% in the first half of the prepatent period. The results suggested that diagnostic sensitivity of *T. s. asiatica* coproantigen detection by sandwich ELISA is higher than the egg examination and proglottid observation during the human experimental infection and it would be useful in diagnosis of the infection.

## **P7 COMPARISON OF DIAGNOSTIC ANTIGENS FOR DETECTION OF CYSTICERCOSIS IN HUMANS, PIGS AND DOGS**

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How to detect individuals infected with metacestodes of *Taenia solium* is very important not only for humans but also for pigs and dogs. We are now using recombinant Ag1V1/Ag2 chimeric antigens (modified from Sako et al. 2000) for both ELISA and Immunoblot as routine diagnosis and its sensitivity and specificity are expected to be better than native antigens (unpublished). In this poster, we use 1) native cyst fluid antigens purified by preparative isoelectric focusing (Ito et al. 1998), 2) those purified by affinity chromatography using polyclonal antibodies against specific glycoproteins and 3) those purified by affinity chromatography using monoclonal antibodies against specific glycoproteins (unpublished), and 4) recombinant Ag1V1/Ag2 chimeric antigens. We also compare a) the quality of the diagnostic antigens from the cyst fluid of *T. solium* metacestodes obtained from Asia, America and Africa and b) the yield of specific antigens from cyst fluid and intact cysts. Serological evaluation of these native antigens and recombinant Ag1V1/Ag2 chimeric antigens is carried out for detection of confirmed and/or suspected cysticercosis cases in humans, pigs and dogs. Both human and pig sera examined are from Asia, America and Africa. Dog sera are from Asia.

## **P8 IMMUNE RESPONSES TO *ECHNOCOCCUS MULTILOCULARIS* INFECTION AND IMMUNE MODULATION BY THE PARASITE IN THE DEFINITIVE HOSTS**

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To reduce the infection pressure to human echinococcosis, novel immune prophylaxis to the definitive host would be effective in addition to deworming of the definitive hosts. Despite the need, the information about immunity to *Echinococcus* infection in the definitive hosts is limited. The humoral and cellular immune responses to the intestinal infection of *E. multilocularis* in dogs and prednisolone-untreated Mongolian gerbils (rodent definitive host model) were investigated. In dogs orally infected with *E. multilocularis* metacestodes (containing ca. 230,000 protoscoleces), serum antibody responses against the parasite antigens were induced from 10 days post-infection (DPI) for IgG1 and from 7 DPI for IgG2, with the highest responses against protoscolex excretory-secretory antigen (PES). Concanavalin A (Con A)-induced proliferative responses of peripheral blood mononuclear cell (PBMC) at 7 DPI decreased remarkably and were restored thereafter. During the 21-day infection period, the lymphocyte proliferative responses to the parasite antigens were not induced in PBMC or the splenocytes at 21 DPI. However, Peyer's patch cells from one of two dogs showed proliferative responses to PES. Con A-induced lymphocyte proliferative responses were suppressed by the addition of parasite antigens. The suppressive effect was remarkable in PES. In gerbils orally inoculated with protoscoleces (ca. 20,000), significant antibody responses (both serum IgG and intestinal IgA) were induced. However, significant lymphocyte proliferative responses to the parasite antigen at 14 DPI were observed only in Peyer's patch cells. The immune suppressive effect of the parasite was assessed by co-cultivation of splenocytes from naive gerbils with mitogens (Con A and LPS) and parasite antigens. Almost all parasite antigens reduced Con A-induced lymphocyte responses in dose-dependent manner, but not LPS-induced responses. The maximum suppressive effect on Con A-induced lymphocyte responses was obtained by adding PES. The characteristics of *E. multilocularis* antigens were examined. The smeared patterns of immunoblotting with serum IgG1 and IgG2 and fecal IgA from the infected dogs indicate that antibodies are produced against carbohydrate moieties of the parasite antigens. High carbohydrate/protein ratio was recognized in PES. Lectin blotting revealed that the parasite antigens consist of  $\alpha$ -D-mannose, N-acetyl-D-glucosamine, sialic acid and sialyl-galactose( $\beta$ 1-3)N-acetyl-D-galactosamine, but little of  $\alpha$ -fucose. The present study first identified that adult *E. multilocularis* possess and secrete mucin-type O-linked glycans. The results indicate that humoral and cellular immune responses are induced by the intestinal infection with the parasites. It is also suggested that the parasites suppress host immune responses by secreting carbohydrate-rich antigen(s) for their establishment and survival in the intestine of the definitive host.

**P9 14-3-3- AND /3-10-GENE EXPRESSION AS MOLECULAR MAKERS FOR THE VIABILITY AND GROWTH ACTIVITY OF LARVAL *ECHINOCOCCUS MULTILOCULARIS***

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Alveolar echinococcosis is a severe, life-threatening parasitic disease caused by massive growth of larval *Echinococcus multilocularis*, developing predominantly in the liver of the intermediate host. The parasite exhibits variable growth activities and proliferation kinetics, which are regulated mainly by the host factors. The aim of this study was to determine whether expression levels of parasite-specific molecules, 14-3-3 and II/3-10, would reflect viability and/or proliferative activity of the parasite. Experimentally, metacestode materials were maintained both *in vivo* and *in vitro* under permissive or non-permissive conditions before the respective gene expression levels were determined by quantitative reverse transcription-PCR. In experiments *in vivo*, transcription levels of the selected molecules in parasite materials were relatively quantified using *beta-actin* as a reference gene. At 2 months after secondary infection, metacestodes recovered from immunodeficient nude mice (BALB/c background) exhibited active growth (mean parasite mass 6.21 g/animal) when compared to the parasite obtained from normal mice (1.85 g/animal). Notably, the massive proliferation of metacestodes in nude mice was clearly associated with a significant increase in 14-3-3 expression level, when compared with the parasite in normal mice. On the other hand, II/3-10 was not over-expressed in the parasite from nude mice whereas metacestodes obtained from normal mice at 2 months post-infection exhibited a significantly lower II/3-10 expression level when compared to the parasite at 1 month post-infection. In experiments *in vitro*, parasites were cultured either under restrictive conditions in the presence of an anti-echinococcal drug nitazoxanide, or under non-restricting control conditions without the drug treatment. Transcription levels of 14-3-3 and II/3-10 decreased during the drug treatment, reaching very low levels by day 8, and they were in parallel with the kinetics of *beta-actin* transcription level. On the other hand, non-treated parasites did not show any significant alterations during the same period of investigation. Immunolocalization of 14-3-3 in *in vitro*-cultured *E. multilocularis* metacestodes revealed that this protein was localized exclusively in proliferative germinal layers. These results suggest that monitoring expression levels of 14-3-3, combined with II/3-10, exhibits a good potential as a molecular tool to evaluate viability and growth activity of larval *E. multilocularis*.

**P10 ANTHELMINTIC BAITING WITH BIOMARKER AGAINST *ECHINOCOCCUS MULTILOCULARIS* IN WILD FOXES IN JAPAN**

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Control studies against *Echinococcus multilocularis* in wild foxes by bait-delivered anthelmintic have been reported in Germany, Switzerland and Japan. In these studies, the changes of the prevalence were evaluated on fox population base, however no evaluation was done on fox individual base. In this study, the bait uptake by foxes and the deworming effect of the foxes taking the baits were evaluated with anthelmintic baits containing biomarker, tetracycline. The study area was the outskirts (110 km<sup>2</sup>) of urban area in Otaru City, Hokkaido, Japan. Biomarker baits containing 50 mg praziquantel and 100-160 mg tetracycline, and non-biomarker baits containing only praziquantel were used. The baits were distributed along roads with 20 baits/km twice from May to August in 2001 & 2002 and 7 times from May to November in 2003 & 2004. A total of 325 foxes captured from June to September in 1999 to 2004 were examined for the parasite with scraping and sedimentation of intestinal mucosa and contents. Tetracycline marking of each fox was also examined using the canine teeth. The proportion of tetracycline-marked foxes was 16 % and 22 % in 2001 and 2002, respectively, when using biomarker baits twice, and 62 % in 2004 when using 4 times. The bait uptake by foxes increased with the frequency of baiting. The proportion of tetracycline marking in juvenile is higher than that in adult foxes. In most tetracycline-marked foxes, *E. multilocularis* were not detected indicating that the foxes eating baits were effectively dewormed. The overall prevalence in foxes in the study area reduced gradually after the bait distribution (from 49 % to 8 %).



**P11 A RETROSPECTIVE STUDY AMONG HYDATIDOSIS PATIENTS IN NEPAL**

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A retrospective survey was conducted between 2 January to 15 April, 2005 to determine the distribution of Hydatidosis patients from Hospitals and Nursing home of Kathmandu valley. Eighty-four Hydatidosis patients were found admitted in which 54.6% were female and 45.4% were male. On the basis of survey records, Hydatid cysts were found in the lung (36.5%), in the liver (30.4%), in the female genital system (21.6%) and spleen as well as other organs (11.5%). In the hospital and Nursing home imaging techniques and serology made the diagnosis. 66.7% patients were treated with surgery, where as 33.3% had oral drug treatment. The mortality rate was 7.1% among the operated cases. Although surgical treatment is widely used in Nepal, where as treatment along with albendazole found effective (57.1%) among patient with non-surgical hydatid cyst.

## P12 THE STUDY OF BIOCHEMICAL PROFILES OF HYDATID CYST FLUID OF ANIMAL AND HUMAN ORIGINS

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Human hydatidosis or cystic echinococcosis is the larval cystic stages of various *Echinococcus* species, and is a major infection having world wide distribution and variable geographical incidence effective control of hydatid disease upon have enough knowledge about transmission. Patterns of disease in endemic area and this with the existence of numerous strain of *Echinococcus granulosus* has been complicated. The present study is to evaluate the biochemical profiles (glucose, urea, creatinine, uric acid, cholesterol, triglycerides, sodium, potassium, calcium, phosphore, magnesium, copper-zinc and total protein) of hydatid cyst fluids form different hosts for the identification of strain variation of *E. granulosus*. Five samples of hydatid fluids were collected from the lung cysts of each host; goats, sheep, cattle, camel, and human. All cyst fluids were centrifuged at 15,000 rpm at 4 °C for 30 min and the supernatants were analyzed for various biochemical parameters by available diagnostic kits. Camel hydatid cyst fluids contained significantly more glucose, urea, creatinie, calcium, protein than other hosts. This difference was statistically significant ( $p<0.05$ ). Quantitative similarities in the biochemical profiles of hydatid fluids of sheep-dog strain of *E. granulosus* in human and other intermediate hosts. This difference of the biochemical parameters of camel cyst with those other species suggests the existence of dog-camel strain in camel.

## **P13 STUDY OF EPIDEMIOLOGY IN EDUCATIONAL MASHHAD'S HOSPITAL IN PATIENTS WITH HYDATID CYST**

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**Introduction & Objective:** Hydatidosis is one of important parasitic zoonosis, which caused by dog tapeworm, *Echinococcus granulosus*. This disease is prevalent in Iran such as other countries, and involves various tissues of the body, specially, liver and lung. The objective of this study is real description of epidemiology conditions of patients that meet hospital in Mashhad. In order to determine method and recommendation in control the disease condition. **Methods:** This survey was a retrospective descriptive, and a cross-sectional study of epidemiology in patients suffered from hydatid cyst, that had been admitted in educational hospitals in Mashhad during the years 1998 - 2003. In this study, condition of age, genus, business, anatomical location of cyst in the body, geographical distribution and type of treatment considered. **Result:** 420 patients were diagnosed as hydatid disease, in 1998-2003. In patients, average of their age group was 35.5 years. The most number of the patients were among the age group of 20-30 years old and the least numbers of them were than 60 - year old patients were female mostly (59%) sex factor in the age group showed that the woman are more affected by hydatid in old ages. The most number of patient were many housekeeper and then farmer, students, respectively, more of patients lived in village (54%). In the study suffered from hydatid cyst of liver mostly (67%) where as prevalence of hydatid of the lung (23%), the most involved organ was the right lung (60.5%) and in the liver right lobe (64.5%) had the most involvement. The most common of chief complaint of patients with hydatid cyst of lung was cough (84%) and in the liver was pain in RUQ (72.5%). A majority of the compatients had single cyst (64%), patients with hydatid cyst were without implicated (58.2%), (15.8%) of them were with velapse of the cyst. Most important treatment was surgical interventions (92.3%) where as only (7.7%) of them were engaged in medical treatments. The average time for hospitalization was  $12.8 \pm 6.6$  days. **Conclusion:** Hydatidosis is a highly invasive, destructive disease. The cysts may take years to produce clinical symptoms. And retrospective survey data on human hydatidosis can give an accurate picture of the prevalence of infection. So, these data remain useful indication of infection prevalence.

**P14 IMMUNIZATION OF SHEEP WITH CRUDE ANTIGEN OF *ECHINOCOCCUS GRANULOSUS***

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Recent evidence points to cystic echinococcosis being a public health problem of increasing concern in a number of countries where control programmes have been reduced due to economic problems and lack of resources, or have yet to be fully investigated. In some parts of the world, some investigators have been developed some effective vaccine against the disease. Because of the importance of this zoonoses in Iran, in current study, two hundreds of *Echinococcus granulosus* adult obtained from Department of Parasitology, School of Veterinary Medicine, Ferdowsi University of Mashhad. Soluble protein of parasite was prepared. The sample was homogenized in a blender, sonicated on ice and then centrifuged for 15min at 10,000 x g. Final yield was kept at -20°C until used. Ten lambs 4-6 months of age of mixed sex, were divided into 2 groups of 5 lambs. Each lamb in test group was vaccinated subcutaneously in the neck with a 2 ml dose of vaccine [1 mg of whole body of *E. granulosus* protein dissolved in 1 ml of phosphate buffer saline (PBS) plus 1 ml of Freund's complete adjuvant (FCA)]. Control lambs were vaccinated with adjuvant in PBS. Lambs were re-vaccinated 2 and 4 weeks after the first vaccination with the same preparation except that FCA was replaced by Freund's incomplete adjuvant (FIA). Three weeks after the second vaccination, each lamb received a challenge infection with 2,000 protoscoleces intraperitoneally and 10 gravid of *E. granulosus*. All sheep were killed after 9 months and examined for hydatid cysts. Results were shown that, in comparison to control lambs, smaller cysts were observed in vaccinated lambs and, in only one of the vaccinated lambs, two and one cyst were observed in liver and lung, respectively. The number of cysts in vaccinated lambs were significantly lower than in control group ( $p < 0.05$ ). This result indicated that the protective immunity in sheep with crude antigen of *E. granulosus* was 90.9%. The protective immunity of the antigen was very high. Our results were along with the studies that have been done by other researchers with the native oncosphere antigen. Results in these studies showed that crude antigen of *E. granulosus* could be a good candidate for vaccination of intermediate host. We are going to detect the protein profiles of this antigen and then test the protective immunity of each pattern in sheep. If the protective immunity was obtained, we will be able to prepare protein (s) in a large-scale for vaccination in sheep.

**P15 A STUDY ON HELMINTH EGGS IN VEGETABLE CONSUMED IN TORBATHEHYDARIEH IN IRAN**

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In the past decade, outbreaks of human illness associated with the consumption of raw vegetables and fruits have increased in the Iran. The presence of pathogenic parasites on fresh fruits and raw vegetables has been extensively documented. Contamination of produce can occur in the field or orchard during harvesting, post harvest, handling, processing, shipping, marketing, and in the home. Two hundred vegetable samples were studied during the period from spring 2003 to winter 2003. Vegetable samples were collected. 100 samples from field and 100 samples from marketing. Samples were examined for helminth eggs. In natural water, both sides of vegetable were washed carefully with a hard brush and the water analyzed by the sedimentation method. The species of parasites were determined. Five species of parasite eggs were found. Of them, 1% *Ascaris lumbricoides*, 0.5 % *Trichostrongylus* sp., 0.5 % *Trichuris* sp., 0.5 % *Hymenolepis nana* and 1% *Taenia* sp. Also 2 % Rhabditoid and filariform Larvae were found.

## **P16 PREVALENCE OF CYSTICERCOSIS IN SLAUGHTERED CAMEL IN MASHHAD (NORTHWEST OF IRAN) DURING 12 MONTHS**

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*Taenia saginata*, the tapeworm is one of the commonest tapeworms of man. The larva of this worm commonly develops in the ox into *Cysticercus bovis*, but it is also reported in camel. A survey was carried out at Mashhad abattoir in order to estimate the incidence of cysticercosis in slaughtered camel during one year. At postmortem inspection of 16,626 camel carcasses, 47 cases were diagnosed as infected with cysticercosis. All of the affected carcasses were slightly infested and passed for consumption after removal of the affected parts and treatment of the by freezing. In laboratory examination of samples, which randomly obtained from affected cases, all samples were diagnosed *C. bovis*. Considering high prevalence of this parasitic infection in Iran, Meat inspection by veterinarians and recommending adequate cooking or freezing of meat has an important role in controlling the infection.

## **P17 PREVALENCE OF *CYSTICERCUS BOVIS* IN POSTMORTEM INSPECTION OF BOVINE CARCASSES IN MASHHAD ABATTOIR**

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The beef tapeworm is an important infection of both humans and cattle. Humans are the only final host and infection is acquired by eating raw or undercooked meat. Where cattle-grazing area is contaminated with human faeces and proglottid or eggs are eaten by cattle and the larvae (cysticerci) will develop in muscles. The cystic forms are most commonly found in the muscles of the heart, masseter, shoulder, diaphragm, tongue, and oesophagus. In a survey at Mashhad abattoir, by incisions of muscles at postmortem inspection of 44,106 cattle carcasses during one year, 310 cases were diagnosed as infected with *Cysticercus bovis*. Nine carcasses were condemned because of extensive infestation and 301 cases were slightly infested and passed for food after removal of the affected parts and treatment of the carcasses by freezing. In this survey, one carcass showed affection of the liver with *C. bovis*, but there was not any cystic form in muscles, and we report it as a rare case. Considering high prevalence of this parasitic infection in Iran, Meat inspection by veterinarians and recommending adequate cooking or freezing of meat has an important role in controlling the infection.

**P18 SEROLOGY AND RISK FACTORS FOR *TAENIA SOLIUM* CYSTICERCOSIS IN INDIAN PATIENTS WITH SINGLE AND MULTIPLE ENHANCING LESIONS**

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To evaluate the serological indicators and risk factors for *Taenia solium* cysticercosis in patients with single and multiple enhancing lesions in India, a total of 129 consecutive patients with seizure disorder attending a general neurology/private clinic and specialized epilepsy clinic were studied and information regarding their food habits (pork consumption), passage of proglottides in faeces and family history of seizure was obtained in addition to a blood sample. Serum samples were analyzed by the presence of *Taenia solium* antibodies by ELISA and immunoblot (Ito et al., 1998). The cut-off optical density for ELISA was 0.06. Of 129 patients with seizure disorder, 64 (49.6%) patients had evidence of [single lesion in 57 (89.1%) and multiple lesion in 7(10.9%) patients, (active or inactive)] neurocysticercosis whilst the remaining 65 (50.4%) patients had no evidence of neurocysticercosis but could have other findings upon imaging. Thirty-five (27.1%) patients reported consumption of pork. Twenty-five (19.4%) who reported pork ingestion had evidence of neurocysticercosis while 10 (7.7%) had no evidence of neurocysticercosis upon imaging. Four (3.1%) patients with history of passage of proglottides in their faeces had evidence of neurocysticercosis. Immunoblot and ELISA were positive in 6 (4.6%) patients. Out of 6 patients reactive in serology, 3 (2.3%) demonstrated single enhancing lesions upon imaging and 3 (2.3%) patients had evidence of multiple neurocysticercosis. Out of 19 (14.7%) patients with family history of seizures, 7 (5.4%) patients had evidence of neurocysticercosis. Pork consumption and passage of proglottides in faeces of intestinal carrier of tapeworm are potential risk factors of developing cysticercosis. Serology has poor yield in patients with evidence of neurocysticercosis, particularly solitary cysticercus granuloma in Indian subcontinent. Speculative reasons for the poor yield will be discussed.



## **P19 ELECTROCLINICAL CHARACTERISTICS OF SEIZURES IN ASSOCIATION WITH NEUROCYSTICERCOSIS IN PATIENTS PRESENTING WITH SINGLE SEIZURE AND PRESUMED NEW PNSET EPILEPSY**

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The occurrence of new onset and recurrent seizures may be associated with imaging findings suggestive of neurocysticercosis in several geographic regions. Electroclinical characteristics of such seizures have not been comprehensively studied, so to determine distinguishing electroclinical characteristics of new onset and recurrent seizures in persons with imaging abnormalities suggestive of single or multiple, active or inactive neurocysticercosis, 308 consecutive persons presenting with a single seizure and 222 consecutive persons with two discrete seizure episodes (designated as presumed newly diagnosed epilepsy), irrespective of whether provoked or unprovoked were divided into 2 subgroups each : (1) those in whom imaging studies revealed abnormalities compatible with a diagnosis of active or inactive neurocysticercosis {n=184 (59.7%) in the single seizure group ; n=95 (42.8%) in the presumed newly diagnosed epilepsy group} and (2) those in whom either imaging was not done due to a firm diagnosis of idiopathic generalized epilepsy, was normal or revealed abnormalities other than being compatible with neurocysticercosis {n=124 (40.3%) in single seizure and n=127 (57.2%) in the presumed newly diagnosed epilepsy group. Detailed clinical electrographic features of seizures in each subgroup were studied and compared using standard statistical tests and in a discriminant model. In the single seizure group, electroclinical features that were significantly associated with imaging diagnosis of neurocysticercosis included focal seizures [specifically somatosensory focal-clonic, focal-tonic and visual seizures ( $p<0.05$ , each)], seizure clusters within 24 hrs ( $p<0.0001$ ), post-ictal neurological deficits ( $p<0.05$ ) and peri-ictal headache ( $p<0.05$ ). A combination of myoclonic and generalized tonic clonic seizures and electroencephalographic findings of focal epileptiform activity ( $p<0.05$ ) and primary generalized epilepsy ( $p<0.0001$ ) were significantly more common in the other (non-neurocysticercosis) subgroup. In the presumed newly diagnosed epilepsy group the occurrence of focal seizures, (including somatosensory ( $p<0.05$ ), focal-clonic ( $p<0.0001$ ), and aphasic seizures ( $p<0.05$ ), seizure clusters within 24, 48 and 72 hrs ( $p<0.01$ ) and post-ictal deficits ( $p<0.01$ ) were significantly more common in the neurocysticercosis subgroup, while a combination of myoclonic and tonic clonic seizures ( $p<0.05$ ), myoclonic seizures alone ( $p<0.05$ ), EEG findings of focal epileptiform activity ( $p<0.05$ ) and primary generalized epilepsy ( $p<0.0001$ ) were significantly more common in the other (non-neurocysticercosis) subgroup. In persons presenting with one or two seizures, the occurrence of focal semiologies such as somatosensory, focal-clonic, focal-tonic, aphasic and visual onsets, seizure clusters, post-ictal neurological deficit and peri-ictal headache suggest the presence of underlying neurocysticercosis. Features that are not consistent with an imaging diagnosis of neurocysticercosis include the occurrence of myoclonic seizures alone or in combination with tonic-clonic seizures and EEG findings of focal epileptiform activity and primary generalized epilepsy.

## P20 THE NATURAL HEARTH OF ECHINOCOCCOSIS IN KYZYL-KUM OF CENTRAL ASIA

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Uzbekistan pertains to regions which are endemic badly on echinococcosis. From facts of F. Nazirov and others (2002) for the last 2 years 8,519 persons were handled by echinococcosis. From hurt people, 14.3-20.0 % was children 0-14 ages, 79.6-85.7 % youths and adult. Infection with echinococcosis turned out to be much more, than men. So, from hurt people, 50.9-54.0 % was women, 46.0-49.1 % - men. 317 persons were hurt by echinococcosis only in Samarkand area for period 1996-2002, including 218 women and 99 men. Echinococcosis also widespreads among animals. Echinococcosis rose among large horned live-stock from 24.3 % in 1990 to 46.2% in 2003 i.e. more in two times. The infection of sheep with echinococcosis formed 45.1 % 15 years ago, but 65.0 % it increases in one and a half times at present time. Infection of goats varied from 8.0 to 12.0 %. The growth of echinococcosis is noted among animals during many years in Kyzyl-kum of Central Asia. For this reason, we have learned possibility of rabbits poisoning by echinococcosis. The 22 rabbits of 3-month-age were used for experiement. Rabbits were divided into 2 experimental groups on 11 heads. With *Echinococcus's* eggs brought from shepherds and dogs "tazi" from facility "Usturt" of Karakalpakstan for poisoning rabbits. Dogs of first experimental group were infected with the eggs of *Echinococcus* from shepherd's dogs, and the second group - from dogs "tazi". Each rabbits were given on 5 thous. copies alive, invasion eggs of *Echinococcus* with a few quantity of water through mouth. The observation was led for rabbits during 3 months. Whereupon, animals were downtrodden and internal organs and heads were researched on echinococcus. After experimental poisoning of rabbits, in 90 days it known by eggs of echinococcus which were revealed from 11 animals infected with material from shepherds' dogs the cenurs were revealed from 8 heads that formed 72.2 %. The signs of contamination were not revealed in 4 heads. The Size of cysts formed to 4.0 sm. and inwardly of cysts were discovered scant few of transparent liquid and protoscolices. From found protoscolices 50-70 were mature i.e. had dark-brown colors transverse drawing. The Rabbits experimental infected with the eggs of *Echinococcus* "tazi" from 11 heads were infected 10 heads that formed 90.0 %. In them was found 105 cenur cysts. The size of cysts reached 6-7 sm., cyst was filled with transparent liquid, and in it was discovered an ensemble of protoscolices. In large cysts an amount to liquids formed 50.0 – 60.0 ml. the walls of cysts were fine, were easy cut. The signs of petrification were not found. At the average in each bubble was noted 1.5- 3.0 thous. copies of protoscolices parasite.

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