XII International Conference on Trichinellosis

BOOK OF ABSTRACTS

Croatia

National Park Plitvice Lakes

25th - 30th September 2007

The 12th International Conference on Trichinellosis

Plitvice Lakes, Croatia
25th – 30th September 2007

www.ict12.info

International Commission for Trichinellosis
Croatian Veterinary Society

PROCEEDINGS
Editors: Tihomir Florijančić, Ras Lužaić, Albert Marinculić

Designed by Ras Lužaić

Printed by TIPO Osijek

XII International Conference
on Trichinellosis

BOOK OF ABSTRACTS

Croatia
National Park Plitvice Lakes
25th – 30th September 2007

TABLE OF CONTENTS

WELCOME ADDRESS

We would like to extend a warm welcome to all the participants of the 12th International Conference on Trichinellosis (ICT12). We expect that the Conference will not only follow the excellent tradition of previous conferences, but that it will also provide all of you with the updates on intriguing Trichinella science.

The 12 th International Conference on Trichinellosis has been jointly organised by the International Commission for Trichinellosis and the Croatian Veterinary Society. The organizers are proud to provide the opportunity for parasitologists, veterinarians and health care professionals from all over the world to present and learn about different problems and various approaches to the disease. We are sure that this will be an enriching experience for all of us.

All program components of the 12th International Conference on Trichinellosis, such as Plenary Lectures, Oral and Poster Presentations, Meetings, ICT Students Award Presentation will hopefully attract to the Lecture Room as many attendees as in previous years. Thank you in advance for your comments, involvement, and expertise. I know this will be a productive conference, generating a lot of good ideas and suggestions for the strategy of the control of the disease.

Abstract submissions exceeded expectation. Overall, a total of 145 abstracts were accepted and will be presented among the Conference.

We would like to express our sincere appreciation to the authors of invited and contributed papers and to all conference participants for their active participation. We also wish to express our heartfelt thanks to the Organizing Committee Members for their substantial contributions to the conference. We are very grateful to all the people who have worked hard in preparing the conference and the Proceedings and making the conference successful.

Finally we would like also to express our gratitude to the sponsors for their great support.

We are delighted to be your host and hope that our ICT12 will enable all of us to share and celebrate our common passion for this intriguing parasite.

Jean Dupouy Camet	Relja Beck	Tihomir Florijančić	Albert Marinculić
President ICT	Program Chair	Secretary	President

COMMITTEES

International Organizing Committee

A. Marinculić (Croatia)
B. Moskwa (Poland)
C. Cretu (Romania)
C. Lawrence (UK)
C. Maddox-Hyttel (Denmark)
D. D. Despommier (USA)
F. Bolas-Fernandez (Spain)
F. Bruschi (Italy)
G. Ortega Pierres (Mexico)
J. Appleton (USA)
J. Posedi (Slovenia)
K. D. Murrell (USA)
L. Forbes (Canada)
L. Sofronić (Serbia)
M. Liu (China)
M. Ribicich (Argentina)
P. Boireau (France)
P. Panichabhongse (Thailand)
R. Blaga (Romania)
Y. van der Giessen (Netherlands)

Local Organizing Committee

A. Marinculić (Chair)
R. Beck (Programme Chair)
T. Florijančić (Secretary)
A. Gašpar
A. Beus
B. Antunović
B. Desnica
H. Pavasović
J. Granić
K. Krešić
M. Šimpraga
Ž. Mišetić
E. Terlević
D. Golubić

ICT Executive Committee

J. Dupouy-Camet – President (France)
H. R. Gamble – Vice-President (USA)
C. M. O. Kapel – Secretary General (Denmark)
E. Pozio – Past President (Italy)
A. Gajadhar (Canada)
K. Noeckler (Germany)
L.Y. Mulia (Mexico)
R. Ko (China)

ACKNOWLEDGMENTS

The organizer gratefully aknowledge the support of the following institutions and sponsor for their significant contribution:

MAJOR SPONSORS

PRIONICS

ELECTRICA FOSSI

SAFE PATH

INEL

OLYMPUS

MEDVETNET

HRVATSKA AGENCIJA ZA HRANU

BIACORE

INSTITUTIONS

MINISTRY OF AGRICULTURE, FORESTRY AND WATER RESOURCES
MINISTRY OF SCIENCE, EDUCATION AND SPORTS
MINISTRY OF HEALTH
FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF ZAGREB

GENERAL INFORMATIONS

The 12th International Conference for Trichinellosis is held at the Hotel Jezero, National Park Plitvice Lakes, Croatia, 25th-30th September 2007.

LANGUAGE
The official language of the Conference is English.

MOBILE PHONE
Participants are advised to switch of the mobile phones during Sessions.

REGISTRATION AND INFORMATION DESK
All kind of informations are available at the desk.
For lost and found personal belongings please ask at the desk.

BADGE
Please, wear always your badge during the conference and social events. If you lose your badge, a new one can be prepared at the Registration Desk.

FIRST AID
In case of emergency, please contact the Registration and Information Desk.

EXHIBITION
The Exhibition is held at the Ground Floor, near the Main Conference Room.

SCIENTIFIC INFORMATIONS

SLIDE PREVIEW ROOM
Slide Preview Room is located at the ground floor near the Main Conference Room.
Please give your presentatioon CDROM (or USB stick) at least two hours before your presentation is scheduled. If it is possible please do this a day before.

POSTER EXHIBITION
All posters are displayed at the Exhibition Hall for two consecutive days. Materials required to mount posters will be available. The maximum height of the poster display board is 2,0 m and the maximum width is 1,0 m.

INSTRUCTIONS FOR POSTER DISPLAY
Posters for Poster Session on Wednesday and Thursday should be mounted at the latest on Wednesday till 10:00 AM left on the board till Thursday 18:00 PM.
Posters for Poster session on Friday and Saturday should be mounted at the latest on Friday till 10:00 AM and left on board till Saturday 18:00 PM.

INSTRUCTIONS FOR POSTER PRESENTATIONS
Presenting authors are asked to attend the Poster session and explain briefly the methods and results of the study in 3 minutes +2 minutes for questions from the audience.

INSTRUCTIONS FOR ORAL PRESENTATIONS
Please give your presentation on CD ROM (or USB stick) in the Slide Preview Room at least two hours before or better the day before the term of the presentation. Speakers are requested to come to the meeting room at least 10 minutes before the start of the Session and identify themselves to the Chairpersons. Speakers have been allocated 10 minutes including discussion (2 minutes).

INSTRUCTION FOR CHAIRPERSONS
Before the Session the Chairpersons have to check at the Registration Desk to see if there are no shows or last minute changes of the program.
Chairpersons must be present in the room 10 minutes before the start of the Session to confirm the presentations.
Chairpersons should instruct the presenters as to the time scheduled to the presentation.

1.

Presidential Address

Trichinella and trichinellosis: recent advances since San Diego meeting

J. Dupoy-Camet

French National reference Centre on Trichinella, Hopital Cochin, Paris Descartes University, 27 Fbrg St Jacques, 75014, Paris, France

Human trichinellosis is still present in eastern Europe, China and Argentina but huge outbreaks have emerged in Turkey and Laos. New foci have been reported in Sardinia & Israel. Backyard pork and hunted meats are still source of these cases. The ICT network (still to enlarge) identified >3000 human cases in 2004. Advances have been made for mass diagnosis (dip-stick method) and for increasing albendazole bio-availability. New proteins interfering with the cell cycle and maintaining the nurse cell have been identified. *Trichinella* is always a fascinating model for immunologists : description of new mediators, chromosomic mapping of host resistance, DNA and encapsulated oral vaccines, antibodies recognising heart specific antigens and inducing myocarditis, identification of early stages antigens. *Trichinella* could alleviate a flu respiratory infection or an autoimmune diabetes in mice. New regulations for meat inspection in the EU and on the efficiency of horsemeat or pork deep-freezing have been discussed and implemented. The ICT board collaborated to "FAO/WHO/OIE Guidelines on the management of human and animal trichinellosis". The european program MedVetNet supported a lot of teams. Two special issues of internationally recognised journals (Vet Parasitology in 2005 and Wiad Parazytol. in 2006) have been devoted to several aspects of the parasite. Finally a major contribution on the evolution of the genus was published by D. Zarlenga in the PNAS (reported by WC Campbell). But here is still a lot to explore: does Trichinella exists in the Amazon basin? mechanisms of neurological complications ? a need for drugs able to destroy encapsulated larvae, role of angiogenesis inhibitors factors in the nurse cell formation ?….

2. The analysis of single larvae reveals a high intra and inter-isolate genetic variability in *Trichinella spiralis*

La Rosa, G., G. Marucci, E. Pozio

Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

In the last years, the development of new technologies, such as the analysis of microsatellite polymorphisms, has increased the opportunity to study the genetic variation as a factor influencing selection, resistance, gene flow and fitness of individuals and populations. Preliminary results have shown that microsatellites are highly variable genetic markers in the genus *Trichinella*, which can be easily identified by PCR-derived methods. This very sensitive and promising tool allows to elucidate the population genetic and the phylogeny. The aim of this work was the genetic analysis of single worms of *Trichinella spiralis* to investigate their allelic polymorphism. To analyze the presence of different alleles in the genome of single larvae, fluorescent primers were synthesized and the amplification products were submitted to ABI 3100 Automated Capillary DNA Sequencer for genotyping. The variation at 12 microsatellite loci was investigated analyzing five single larvae of 16 isolates belonging to different geographical regions. The analysis has revealed a remarkable amount of genetic diversity both intra-isolate and inter-isolates. This unexpected genetic variability opens new scenarios to the study of the gene flow in *T. spiralis*, allowing us to understand the complex transmission mechanisms. At the same time, the genotyping of single isolates will be an useful tool to establish a barcoding for *T. spiralis* isolates.

3. Identification of *Trichinella* Spanish isolates by ISSR-PCR: Intra-specific variability of *Trichinella britovi*

J. Perteguer, María, E. Rodríguez, R.N. García-Sánchez, J.J. Nogal-Ruiz, F. Bolas-Fernandez, A.R. Martínez-Fernández, T. Gárate

Departamento de Parasitología. Facultad de Farmacia, UCM, Madrid, Spain.

In Spain, trichinellosis represents a public health problem with an average of five outbreaks/year reported, being pork and wild boar meat the main sources of infection; It occurs as sylvatic and/or domestic cycle with two *Trichinella* species implicated, *Trichinella spiralis* and *Trichinella britovi*, which show identical morphology. *T. spiralis* is widespread in both domestic and wild animals, while *T. britovi* is mainly responsible of the sylvatic cycle. In the present work, we have investigated the variability of the *Trichinella* Spanish isolates by ISSR-PCR (inter-simple sequence repeat polymerase chain reaction), a technique that is being successfully used to study genetic diversity among related populations. We analized a total of 43 isolates from different geographic localization by Multiplex-PCR and ISSR-PCR. 19 out of 43 isolates (44.2%) showed a pattern similar to the *T. spiralis* (ISS116) reference strain pattern using both techniques. All the *T. spiralis* isolates examined were recovered from wild boar (*Sus scrofa fera*) except five pig isolates (*Sus scrofa domestica*). The other 25 isolates were identified as *T. britovi* (55.8%), which showed two banding patterns compatible with the *T. britovi* reference strain pattern (ISS2), and the *T. britovi* autochthonous isolate (ISS11), 95.8% and 4.2% respectively. *T. britovi* isolates were obtained from wild boar (*Sus scrofa fera*) in all the cases except two, corresponding to a fox isolate (*Vulpes vulpes*) and to a pig isolate (*Sus scrofa domestica*).

This work was supported by TRICHIMED grant (MPY13/04-27) of the MED-VET-NET project (FOOD-CT-2004 506122) and by the RETIC project (RICET; RD06/0021/0019).

María J. Perteguer
Instituto de Salud Carlos III,
Department of Parasitología
Centro Nacional de Microbiología
Ctra. Majadahonda-Pozuelo Km. 2,2
28222 Majadahonda
Madrid/Spain
Phone: +34918223603
e-mail: chus.perteguer@isciii.es

4. Molecular characterization of non-encapsulated *Trichinella* species by an inter-simple sequence repeat polymerase chain reaction (ISSR-PCR)

Martínez-Fernández, A.R[1], R.N. García-Sánchez[1], J.J. Nogal-Ruiz[1], F. Bolas-Fernandez[1], E. Rodríguez[1], M.J. Perteguer[1], E. Pozio[2], T. Gárate[3]

[1] Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III. Majadahonda, 28220 Madrid, Spain.
[2] Dept. Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanitŕ, Rome, Italy.
[3]Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III. Majadahonda, 28220 Madrid, Spain.

The inter-simple sequence repeat polymerase chain reaction (ISSR-PCR) allows the amplification of mono, di, tri, or tetranucleotide motif repeats in multiple tandem copies. This technique provides a quick, reliable, and highly informative system for DNA banding patterns that permits inter- and intra- species identification. The method has been previously standardized for encapsulated *Trichinella* species and for the non-encapsulated *Trichinella pseudospiralis* in our laboratory. The aim of the present work is to characterize non-encapsulated *Trichinella* species by ISSR-PCR. According to the different zoogeographical regions the following *T. pseudospiralis* isolates were studied: (i) Palearctic region: Caucasus (ISS13), Kazhastan (ISS176), Kamchatka (ISS588); (ii) Neartic region: Alabama (ISS470); and (iii) Australian region: Tasmania (ISS141). In addition, other two non-encapsulated *Trichinella* species were investigated, *Trichinella papuae* from Papua New Guinea (ISS572) and *Trichinella zimbabwensis* from Zimbabwe (ISS1029). The primer 816 (5' CACACACACACACACAT 3') allowed the distinction of the five different *T. pseudospiralis* isolates and the two species *T. papuae* and *T. zimbabwensis*. The similarity relationships among the *Trichinella* isolates examined were depicted in a dendrogram using the UPGMA algorithm. The *T. pseudospiralis* isolates were grouped together, being further subdivided into two clusters: (1) the Paleartic and Australian isolates; and (2) the Neartic isolate. Another cluster included the species *T. papuae* and *T. zimbabwensis* that were closely related but separated from the *T. pseudospiralis* cluster. ISSR-PCR emerges as an useful technique to study the genetic variability in the *Trichinella* genus.

This work was supported by TRICHIMED grant (MPY13/04-27) of the MED-VET-NET project (FOOD-CT-2004 506122) and by the RETIC project (RICET; RD06/0021/0019).

Antonio R. Martínez-Fernández
Universidad Complutense de Madrid
Parasitología, Facultad de Farmacia
Plaza de Ramón y Cajal s/n
282040 Madrid/Spain
Phone: +34913941817
e-mail: arm@farm.ucm.es

5. Typing of a new emcapsulated *Trichinella* genotype in a wild boar from Navarra (Spain)

Martínez-Fernández, A. R.[1], J.J. Nogal-Ruiz[1], R.N. García-Sánchez[1], X. Gerrikagoitia[2], M. Barral [2]

[1]Faculty of Pharmacy. Complutense University, Department of Parasitology, Pza. Ramón y Cajal s/n, Madrid, Spain
[2]Instituto Vasco de Investigación y Desarrollo Agrario (NEIKER). Departamento de Sanidad Animal. Derio (Vizcaya), Spain

A trichinellosis survey was carried out in the context of a surveillance program on wildlife diseases in the Basque Country and Navarra (Spain). Samples from diaphragm, tongue, masseter, intercostals and foreleg were examined by trichinoscopy and/or digestion. The larvae recovered were submitted to molecular typing by ISS-PCR for species identification. In one sample from a wild boar cysts with an atypical morphology surrounding the larvae were found. The banding pattern of these larvae following ISSR-PCR analysis was different from those obtained for the reference strains: *T. spiralis* (ISS48), two *T. britovi* (ISS11 and ISS2), genotype T8 (ISS48), genotype T6 (ISS34), *T. murrelli* (ISS35), *T. nelsoni* (ISS29) and *T. nativa* (ISS10). Application of the NTSYSpc software to the banding pattern obtained by molecular typing allowed the construction of trees of similarity and minimum-length spanning. In all cases the new genotype called "suisiberica" (MSUS/SP/07) plotted close to the genotype T6, being fairly separated from *T. spiralis*, *T. native, T. murrelli* and *T. nelsoni*.

Morphological observation under compression showed cysts with brown colour and dense appearance. Histological examination following H&E staining evidenced well delimited cysts with strong collagen capsule and light inflammatory reaction surrounding the cyst wall. The larvae looked bigger than usual, with a normal appearance within the disorganised nurse cell. The free larvae fixed in alcohol have long pharynx and less conspicuous stichosome. The genital primordia were well delimited allowing a clear distinction between male and female.

This work was supported by TRICHIMED: MED-VET-NET (FOOD-CT-2004 506122)

Antonio R. Martínez-Fernández, PhD, Professor
Faculty of Pharmacy
Complutense University
Department of Parasitology
Pza. Ramón y Cajal s/n
28040 Madrid, Spain
Phone: 34 91 394 1814
e-mail: arm@farm.ucm.es

6. A new variety of *Trichinella britovi* present in the Iberian Peninsula wild fauna

García-Sánchez R.N.[1], J.J. Nogal-Ruiz[1], F. Bolas-Fernandez[1], F. Fonseca-Salamanca[1], S. Jimenez[1], R. Álamo[2], A.R. Martínez-Fernández[3]

[1]Consejería de Sanidad y Bienestar Social, Junta de Castilla y León, 47071 Valladolid, Spain
[2]Departamento de Ciencias Preclínicas, Facultad de Medicina, Universidad de La Frontera, Casilla 54-D, Temuco, Chile
[3]Consejería de Salud y Servicios Sociales, Logroño, La Rioja, Spain

In the wild fauna of the Iberian Peninsula *Trichinella spiralis* and *T. britovi* live in apparent sympatry. In the present study 150 isolates from wild mammals were typed by applying the inter-sequence simple repeats ISSR-PCR molecular technique and the results show that all isolates identified as *T. spiralis* were indistinguishable from the ISS48 reference isolate whereas four variations were clearly distinguished among those belonging the *T. britovi*: two of them (ISS11C-76 and ISS86MON) were previously detected and the others (ISS2 and MVUL/SP/02/R3) have not been reported before. Among all them the ISS11 and ISS2 isolates were found to be the most frequent. Collective analysis of ISSR-PCR banding patterns using the NTSYSpc system including these isolates, the genotypes T8 and T9, *T. murrelli*, with or without *T. spiralis* as out-group, allowed the construction of trees of similarity and minimum-length spanning. This analysis suggests the identity and hypothetical relationships of these Trichinella populations. All varieties including the genotype T8 (ISS49) were interfertile by single larva cross-breeding suggesting that they belong to the same species. The uniformity found within *T. spiralis* isolates may indicate its unique and recent introduction whereas the presence of 4 variations within *T. britovi* suggest that this species represents one of the original endemic *Trichinella* in the Paleartic temperate climate, with higher density in the West-End of Eurasia. Orographical diversity of this region would preserve its population variation.

This work was supported by TRICHIMED: MED-VET-NET (FOOD-CT-2004 506122)

Rory Nelson García-Sánchez (Predoctoral felow)
Universidad Nacional de la Amazonía Peruana
Laboratorio de Investigación de Productos Naturales Antiparasitarios de la Amazonía
Complutense University
Pharmacy Faculty
Plaza de Ramón y Cajal s/n
28040 Madrid, Spain
Phone: 34 91 394 1818
e-mail: rgbiounap@yahoo.es

7.

The phylogeny and genome of *Trichinella* species

Zarlenga, D.[1], Makedonka Mitreva[2], B. Rosenthal[3], E. Hoberg[3]

[1]USDA, ARS, ANRI, Department of Bovine Functional Genomics Lab & Animal Parasitic Diseases Lab
[2]Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO 63108 USA
[3]USDA, ARS, ANRI, Animal Parasitic Diseases Lab, Beltsville, MD 20705 USA

In 2004, funding was received by Washington University's Genome Sequencing Center through NHGRI, to completely sequence several nematode genomes as part of a holistic effort to advance our understanding of the human genome. *Trichinella spiralis* was among this group because of its strategic location at the base of the Nematoda phylum, and the belief that extant species represented a divergent event that occurred as early as the Paleozoic. At the same time, a concerted effort was put forth to solidify the phylogeny of extant species of *Trichinella* based upon molecular analyses of a multi-gene system in order to form a framework for understanding the history of the genus and thereby enhance utilization of the forthcoming sequence data. Since the inception of this research, several findings have surfaced; 1) contrary to conventional belief, extant species of *Trichinella* probably diverged as little as 20 million years ago; 2) the genome size of *T. spiralis* (71.3 Mb) is substantially smaller than originally predicted (270 Mb); 3) the 3.5 million sequence reads assembled into a 59.3 Mb unique sequence, of which 19% comprise repetitive elements and; 4) expansion of the EST and genome sequence database confirmed that *T. spiralis* shares a similar proportion (45%) of its ESTs with the nematode Caenorhabditis elegans (Rhabditina) as it does with the fruitfly Drosophila melanogaster (Arthropoda: Drosophilidae). Updated information on the sequencing effort will be presented.

Dante Zarlenga, PhD.
USDA, ARS, ANRI
Department of Bovine Functional Genomics Lab & Animal Parasitic Diseases Lab
Bldg 1180 BARC-East
20705 Beltsville, MD USA
Phone: (301) 504-8754
e-mail: Dante.Zarlenga@ars.usda.gov

8. Early Identification of *Trichinella* Larvae by Polymerase Chain Reaction in Muscle & Blood of Infected Mice

Semont, Karine, A. De Bruyne, Hélčne Yera, T. Ancelle, J. Dupouy-Camet

PARIS DESCARTES UNIVERSITY, TRICHINELLA NATIONAL REFERENCE CENTER, HOPITAL COCHIN APHP, 27 Fbrg St Jacques, Paris, France

We carried out an experimental study with OF1 mice infected by 100 larvae of *Trichinella spiralis* (ISS 104) to compare the sera detection of anti-*Trichinella* antibodies by ELISA, blood detection of newborn larvae by a PCR-based assay targeting the 5S ribosomal DNA intergenic spacer region and larvae detection in muscular biopsies. We detected the presence of DNA in mouse blood from day 9 to day 15 of infection, whereas at 30 days post infection (dpi) the detection of specific antibodies remained negative. The direct examination of muscle biopsies allowed to visualise larvae from 18 dpi, but the DNA could be detected by PCR 8 dpi. These results suggest that parasitic DNA could be detected in muscles a short time after infection at a stage where the microscopic examination is still negative. It could be then, theoretically, possible to type isolates very early after infection by sequencing the amplified DNAs obtained from muscular biopsies but also from blood.

Jean DUPOUY-CAMET, PhD
PARIS DESCARTES UNIVERSITY
TRICHINELLA NATIONAL REFERENCE CENTER
HOPITAL COCHIN APHP
27 Fbrg St Jacques
75014, Paris
France
Phone: 33 1 58 41 22 49
e-mail: jean.dupouy-camet@cch.aphp.fr
Web: http://monsite.wanadoo.fr/cnrdestrichinella/

9. Species identification of *Trichinella* isolates from China

Baoquan, FU, LI Wenhuia, LI Hanga, LI Yongguanga, GAI Wenyana, GOU Huitiana, Wang Yanhuaa, Zhang Delinga, DOU Lanqinga, Liu MingYuanb, R. Blaga, P. Boireau

Trichinellosis, a disease caused by the nematode *Trichinella*, occurs in more than 150 animal species in areas with different geographical and ecological characteristics. In China, *Trichinella* infection in animal is widespread, although little is known about the existing *Trichinella* species genetic variation. By amplification of different target regions (expansion segment V, 5S ribosomal DNA intergenic spacer and mitochondrial small and large subunit ribosomal RNA) by means of PCR, two *Trichinella* species were identified in China. In Henan, Tianjing, Haerbing (North of China), Xian (West of China) and Yunnan (South of China) Trichinella spiralis was identified in five isolates originating from domestic pig, while *Trichinella nativa* was identified in Jilin province (North-East of China), in a dog isolate. The present results confirmed the previous identification of only two *Trichinella* species in mainland China. Furthermore, the sequence analysis of the 5S ribosomal DNA intergenic spacer for the *Trichinella spiralis* isolates indicates the existence of a genetic variation among them.

Work founded by the National Natural Science Foundation of China (NSFC 30328020) and AFCRST (PRA BT 03-02).

Baoquan FU, Professor
Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences
Department of Parasitology
Xujiaping 1, Yanchangbao,
Post Code: 730046 Lanzhou P.R. China
Phone: 86-931-8342675
e-mail: fubaoquan@163.com

10. Identification of six *Trichienlla* isolates from China by multiplex PCR

Cui, J. , G.H. Zhao, Z.Q. Wang, H.T. Niu, P. Jiang

To identify and classify six *Trichinella* isolates from China, the specific five pairs of primers were synthesized based on the DNA sequence of the expansion segment V region and the internal transcribed spacers ITS1 and ITS2 of the ribosomal DNA repeat from *Trichinella*. The International Reference strains of five species of Trichinella were used as control, six *Trichinella* isolates from China were identified by multiplex PCR and its effecting factors of PCR amplification were observed. The results of electrophoresis of multiplex PCR products of *Trichinella* isolates showed that the band (173bp) from swine Trichinella isolates from Henna, Yunnan, Harbin, Tongjiang in Heilongjian, Hubei and Tianjin of China was the same as T. spiralis . The specific band (173bp) of single T. spiralis larva, larvae conserved in 80% ethanol for 6 months, the larvae stored in formalin, 5% formalin, 0.2% sodium azide and 0.05% merthiotate for 2 wk, and the fresh mouse muscle with larvae was amplified by multiplex PCR. The results showed all of six Trichinella isolates from China are identified as *T. spiralis* by multiplex PCR and the method is sensitive and specific for the identification of *Trichinella* genus.

The work was supported by the National Natural Science Foundation of China (30671834) and the Key Project of Science and Technology Plan of Henan Province (072102310014) .

J Cui, Professor
Medical College, Zhengzhou University
Department of Parasitology
Daxue Road 40
450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: cuij@zzu.edu.cn

11. PCR-RFLP patterns of four isolates of *Trichinella* in dogs of Zacatecas, Mexico

Jiménez-Cardoso, E, R.S. Sánchez-Pérez, M.L. Caballero-García, A. Moreno

The variability of the genus *Trichinella* has been reported in terms of morphology, virulence, infectivity, antigenicity and genetic polymorphism. The identification of the sequence regions in the genomes of pathogens which can be useful to distinguish among species and genotypes, is of the great importance for epidemiological, molecular, and phylogenetic studies. The aim of the present study was used the RT-PCR analysis of the 5S and ITS1 rDNA and examined the patterns of four isolates of *Trichinella´s* dogs of Zacatecas, Mexico. The isolates of *Trichinella* were maintained routinely in the laboratory by serial passages thought Wistar rats. Infective state larvae from skeletal muscles of rat were frozen in liquid nitrogen and homogenized in three volumes of Trizol. After centrifugation, RNA was precipited by adding an equal volume of ice-cold isopropanol. The total RNA was suspended in DEPEC treated water. Single-stranded cDNA was synthesized by Reverse Transcription reagents. cDNA generated of the four isolates' s dogs of *Trichinella* were amplified using respective primers and digested with restriction enzymes. Characteristic patterns were produce when samples within four isolates were analyzed. This method offers and objective, simple, highly sensitive and rapid approach for the discrimination of Trichinella isolates. The restriction fragments of each isolate had variable patterns. The estimate genetic divergence between each isolate was different.

Enedina Jimenez Cardoso
Hospital Infantil de Mexico Federico Gomez, Jefe del Laboratorio de Investigacion en Parasitologia
Department of Laboratorio de Investigacion en Parasitologia
Dr Marquez 162, Col. Doctores
06720 Mexico D F
Phone: 55 55884019
e-mail: enedina@servidor.unam.mx

12. Molecular identification of *Trichinella species* circulating among wild and domestic animals in Romania

Blaga, R.[1], C. Gherman[1], V. Cozma[1], A. Glemarec[2], E. Pozio[3], P. Boireau[2]

[1]Faculty of Veterinary Medicine, Parasitology Unit, University of Agricultural Sciences and Veterinary Medicine
[2]JRU BIPAR INRA, AFSSA, ENVA, UPVM, 23 avenue du Général de Gaulle BP 67– 94703 MAISONS-ALFORT cedex, France
[3]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanita, Rome, Italy

Trichinellosis, a food-borne parasitic zoonosis, which affects more than 11 million people worlwide (Dupouy-Camet, 2000), is one of the most serious helminthic zoonosis of Romania. In spite of the presence of both a domestic and a sylvatic cycle, all *Trichinella* spp. isolates identified so far were assumed to be *Trichinella spiralis*. Therefore the present study was conducted to identify *Trichinella* spp. circulating among wild and domestic animals, by PCR-based methods. The potential influence of climate on the distribution of *Trichinella* species was also evaluated. *Trichinella* sp. larvae were detected in 54 (31.8%) of the 147 wild and 23 domestic mammals examined. No *Trichinella* sp. larva was detected in the breast muscle samples of the 182 birds examined. *Trichinella spiralis* and *Trichinella britovi* were the only two species identified in the 40 isolates which showed a positive PCR amplification. *Trichinella britovi* is the most prevalent species (n=26, 65%), followed by *T. spiralis* (n=14; 35%) which was predominant among domestic animals (n=9; 75%), while T. britovi was prevalent among wildlife isolates (n=24; 85.7%). No mixed infection was found. The distribution of *Trichinella* species in Romania does not show a species-specific cluster, like described in Bulgaria, both species being present over the entire examined counties. The highest prevalence of *Trichinella* infection was detected in wolves (31.4%; 11/35), followed by the European wild cats (14.3%; 4/28) and red foxes (7.0%; 5/71).

Work founded in part by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156) and in part by the EU project MedVetNet (Workpackages TRICHINET and TRICHIMED).

Radu Blaga, DVM, PhD
University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine
Department of Parasitology
JRU BIPAR INRA AFSSA ENVA UPVM, 23, avenue du Général de Gaulle BP 67– 94703 MAISONS-ALFORT cedex, France
calea Manastur nr 3-5
400372 Cluj-Napoca ROMANIA
Phone: +40264596384
e-mail: blecgec@yahoo.com

13. Molecular evidence for a novel encapsulated genotype of *Trichinella* from Patagonia, Argentina

Krivokapich, S.[1], Cinthia Gonzalez Prous[1], Graciana Gatti[1], Viviana Molina[1], H. Matarasso[2], E. Guarnera[1]

[1]INEI, ANLIS "Dr. Carlos G. Malbrán", Laboratory of Trichinella and Trichinellosis, Department of Parasitology, Buenos Aires, Argentina
[2] Ministerio de Producción, Vicente López y PLanes 407, (8361), Luis Beltrán, Río Negro, Argentina

At the present, *Trichinela spiralis* is the only species of this genus reported from South America. Herein, we detail a molecular analysis of a new encapsulated isolate of muscle larvae of *Trichinella*, found in a mountain lion (Felis concolor,) coming from the Patagonia (Río Negro, Argentina). We studied three DNA regions that had been recognised to be useful in identification at species level in *Trichinella*: expansion segment 5 (ES5), cytochrome c-oxidase subunit I (COI) and 5S ribosomal DNA intergenic spacer region (5S rDNA ISR). The GenBank database comparison by BLAST search shows that the sequences from ES5, COI and 5S rDNA ISR are different from the eleven currently recognized genotypes of *Trichinella*, although all of each these three sequences reveals the highest homology score with the members of genus *Trichinella*. The phylogenetic analysis of COI and 5S rDNA ISR shows that mountain lion isolate grouping within encapsulated members, in agreement with morphological data, but is not clustered with some genotypes within this encapsulated group. Unfortunately, the larvae of *Trichinella*, coming from the mountain lion were all non-viable and, consequently, could not be done biological and biochemical tests. Nevertheless, this molecular analysis suggest strongly that this sylvatic isolate from the Patagonia correspond to a novel encapsulated genotype of Trichinella.

Silvio Krivokapich, Licenciado en Genética (Geneticist)
INEI, ANLIS "Dr. Carlos G. Malbrán"
Laboratory of Trichinella and Trichinellosis
Department of Parasitology
Av. Velez Sarsfield 563
1281 Buenos Aires Argentina
Phone: 54-11-4301-7437
e-mail: silkri@anlis.gov.ar

14. The neutrophil activating protein, a Th1 adjuvant, modulates the Th2 response in *Trichinella spiralis* infected mice

Bruschi, F.[1], Maria Piazza[1], Lorena Chiumiento[1], M. Masetti[2], S. Mazzoni[1], A. Amedei[3], M.M. D'Elios[3], Andrea Cappon[4], Marina de Bernard[4], G. Del Prete[3]

[1]Dipartimento di Patologia sperimentale, B.M.I.E., Universita di Pisa, Scuola Medica, Pisa, Italy
[2]Dipartimento di Bilogia, Universita di Pisa, Pisa, Italy
[3]Dipartimento Medicina Interna, Universitŕ di Firenze, Firenze, Italy
[4]Venetian Institute of Molecular Medicine, Padova,Italy

The neutrophil activating protein (NAP) of *Helicobacter pylori* is a Toll-like receptor-2 agonist able to induce IL-12 and IL-23 production by monocytes and neutrophils, and to shift in vitro T-cell responses from Th2 into Th0/Th1. To challenge in vivo the Th1 adjuvanticity of recombinant (r) NAP, a well known model of Th2 activation was used: the *Trichinella spiralis* infection. T. spiralis infected BALB/c mice were treated with rNAP in two different protocols: 1) day 0, oral infection with 300 T. spiralis muscle larvae (ML), day 28 post infection (dpi) treatment with rNAP (10 ug) or saline i.p., day 56 sacrifice; 2) day 0 oral infection with 300 T. spiralis ML, dpi 10 and 28 treatment with rNAP (10 ug) or saline i.p., day 56 sacrifice.

At different times of infection blood samples were collected to evaluate total leukocyte (TL), neutrophil (N), eosinophil (E), lymphocyte (L) counts and total IgE (tIgE) levels which were measured with a commercial kit.

Results. Using protocol 1), compared to untreated mice, rNAP-treated mice showed a significant decrease of TL, due to Lymphocyte (42 and 56 dpi) and Eosinophil (56 dpi) reduction. Also tIgE levels were significantly lower upon rNAP treatment. With protocol 2) we observed again a significant decrease of TL, due to L (14 and 42 dpi) and E (28 and 42 dpi) reduction. The mean of tIgE levels in rNAP-treatd mice was lower than in control mice, but poorly significant.

The results will be discussed in the light of host-parasite relations.

Fabrizio Bruschi, Professor
Universita' di Pisa
Department of Experimental Pathology, M.B.I.E.
Via Roma 55
56126 Pisa ITALY
Phone: +39(050)2218547
e-mail: fbruschi@med.unipi.it

15. Isolation, biochemical analysis and localization of a 24kDa glutathione s-transferase from *Trichinella*

Bahuon, Celine[1], R. Blaga[1], BaoQuan Fu[2], F. Bruschi[3], MingYuan Liu[4], Danielle Le Rhun[1], A. Glemarec[1], P. Boireau[1]

[1]JRU BIPAR INRA AFSSA ENVA UPVM, MAISONS-ALFORT cedex, France
[2]Key Laboratory of Veterinary Parasitology of Gansu Province, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, P. R. China
[3]Department of Experimental Pathology, M.B.I.E, University of Pisa, Pisa, Italy
4Key Laboratory of Zoonoses, Ministry of Education, Institute of Zoonoses, Jilin University, Changchun, P. R. China

Survival of *Trichinella* sp., a pathogenic nematode parasite, is likely dependent upon the detoxification activities of the glutathione S-transferases (GSTs). A DNA sequence similar to the GST gene family was first identified from a subtracted cDNA library of Trichinella spiralis new born larvae (NBL) by suppression subtractive hybridization, and was used as a probe for the screening of a *Trichinella spiralis* adult worm with hatched larvae (Ad5) cDNA library. The identified clones shared 47% to 52% of identity with the 24 kDa pi class intracellular GSTs of other nematodes. Moreover, the comparison of *Trichinella spiralis* GST sequence (tsgst24) with those amplified from *Trichinella britovi* (tbgst24) or *Trichinella nativa* (tngst24) revealed a high interspecies nucleotide conservation (96% of identity). The GST of *Trichinella pseudospiralis* (tpsgst24) presented 88% identity with tsgst24. The open reading frame coding for Trichinella spiralis GST24 was expressed in a prokaryotic system and the soluble recombinant protein was purified by glutathione-affinity columns. TsGST24 enzymatic activity was proved to be different from the activity of mammalian pi class GSTs, and other pi class related nematode GSTs. The distribution of TsGST24 was examined by immunohistochemistry, using the anti-GST antibody, in *Trichinella spiralis* muscle larvae (ML) and infected tissues. TsGST24 was located in the larval stichocytes and in the genital primordium. The nurse cell was completely unstained, suggesting that Ts GST is not vehiculed outside the parasite.
Work founded by the EU project TRICHINET/ TRICHIMED workpackage (MedVetNet).

RADU BLAGA, DVM, PhD
University of Agricultural Sciences and Veterinary Medicine, Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort, France
Department of Parasitology Unit, Faculty of Veterinary Medicine
3-5 CALEA MANASTUR
400372 CLUJ-NAPOCA ROMANIA
Phone: 0040264596384
e-mail: blecgec@yahoo.com

16. *Trichinella spiralis* reinfection: cellular immune response in mice

Dvorožňáková, Emília[1], Marta Kolodziej-Sobocińska[2], Zuzana Hurníková[1], Ewa Dziemian[2]

[1]Parasitological Institute SAS, Košice, Slovak Republic
[2]Institute of Parasitology PAS, Warsaw, Poland

Trichinella spiralis infection induces the host cell-mediated and humoral response. The role of Th-lymphocytes and macrophages in the immune response of mice reinfected with 2x400 T. spiralis larvae was studied in relation to the parasite burden. Mice BALB/c were infected on days 0 and 60 and immunological parametres were examined within a period of 180 days. In comparison with single *T. spiralis* infection, T- and B-lymphocytes in reinfected mice responded by a significant increase in the proliferative activity during 10 days after reinfection. At the same time the numbers of CD4 T-cells of reinfected mice were also increased. On the contrary, the CD8 T-cell numbers were significantly reduced almost 30 days after reinfection. High concentration of serum IFN-gamma lasted till the end of the experiment. The IL-5 level was increased only for 2 weeks after reinfection, followed by its fall down. The greatest numbers of peritoneal macrophages were found till day 30 after reinfection along with the maximal production of superoxide anion in macrophages and the generation of nitric oxide by macrophages after reinfection was inhibited. Lower numbers of adults (40.6 % reduction) in the small intestine and 61.4 % reduction in muscle larvae was found after reinfection. Stimulation of cellular immunity after reinfection contributed for the reduction of the parasite burden.

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-51-010704 and Slovak Research and Development Agency under the contract No. APVV-51-027605.

Emilia Dvorožňáková, DVM
Parasitological Institute SAS
Hlinkova 3, 040 01 Košice
Slovak Republic
Phone: ++421 55 6334455
e-mail: dvoroz@saske.sk

17. Proteomic analysis of *Trichinella spiralis* L1 muscle larvae under different culture conditions

Bolás-Fernández, F., M. Robinson, María Auxiliadora Dea-Ayuela, Bernadette Connolly

Complutense University, Faculty of Pharmacy, Department of Parasitology, Plaza de Ramón y Cajal s/n, Ciudad Universitaria, Madrid, Spain

After digestion of infected meat the free L1 larvae of *Trichinella* spp penetrate the intestinal mucosa where they undergo molting to reach mature adult stages within little more than 24 hours. Molting and ecdysis is a complex physiological process shared by nematodes and arthropods which is orchestrated by the combination of multiple physio-chemical environmental factors. In the present paper, we have taken a proteomic approach to identify changes in protein secretion during culture of free *T.spiralis* muscle larvae under different environmental conditions and to correlate these changes with their infectivity in mice. L1 larvae were cultured in 3 different media (RPMI-1640, C-199 and HBSS) under anaerobiosis, microaerobiosis and 5% CO2, with or without HEPES, at 37şC for 20, 24 and 28 hours. Following incubation, the *Trichinella* larvae were used to orally infect nad'ye CD1 mice and the larval secretory proteins were precipitated from the culture media and analysed by 2-dimensional gel electrophoresis in comparison with a 2-D reference map. Anaerobiosis in all media or microaerobiosis in HBSS preserved larval infectivity whereas microaerobiosis in RPMI-1640 and C-199 decreased infectivity in a larva concentration-dependent manner. Comparative analysis of larval infectivity and protein secretion showed that, over all, infectivity is associated with the presence/absence of non-tyvelosilated proteins (such as MCD-1, 5'-nucleotidase, ORF.11.30, ORFF17.20, BG520944 and the BG353021 group). These results suggest that non-tyvelosylated proteins secreted following larval activation by micro-environmental factors such as nutrient availability and CO2 concentration may play a role in infectivity, particularly during the molting process.

Francisco Bolás-Fernández, PhD
Complutense University
Faculty of Pharmacy
Department of Parasitology
Plaza de Ramón y Cajal s/n, Ciudad Universitaria
28040 Madrid, Spain
Phone: +34913941818
e-mail: francisb@farm.ucm.es

18. Effect of phytohaemagglutinin-P on $CD4^+$ and $CD8^+$ T cells apoptosis in *Trichinella spiralis* infected mice

Piekarska, Jolanta[1], Szczypka Marianna[2], M. Gorczykowski[3], Bożena Obmińska-Mrukowicz[3] and Monika Pawlas-Opiela[3]

[1]Department of Internal and Parasitic Diseases,Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Pl.Grunwaldzki 47, Poland
[2]Department of Biochemistry, Pharmacology and Toxicology,Faculty of Veterinary Medicine,Wroclaw University of Environmental and Life Sciences, ul. Norwida 31, Poland
[3]Department of Biochemistry,Pharmacology and Toxicology,Faculty of Veterinary Medicine,Wroclaw University of Environmental and Life Sciences,ul. Norwida 31, Poland

Flow cytometry analyses were used to determine the presence and phenotype of apoptotic lymphocytes in *Trichinella spiralis*-infected mice, treated with phytohaemagglutinin-P (PHA-P), 24h before infection. The percentage of $CD4^+$ and $CD8^+$ in the spleen, mesenteric lymph node, and muscular inflammatory infiltration cells were described on 7, 14, 21, 28, 35, 60 day post infection (dpi) using monoclonal antibodies and Annexin-V-Fluos Staining Kit. In *T. spiralis* - infected mice, the highest percentage of apoptotic $CD4^+$ cells was found on 14 dpi in the mesenteric lymph nodes (21,8%), on 21 dpi in the spleen (3%) and in the muscular inflammatory infiltration (4%). The peak of apoptotic $CD8^+$ cells was found on 14 dpi in the mesenteric lymph nodes (13,3%), on 28 dpi in the spleen (2,8%) and 35 dpi in the muscular inflammatory infiltration (1,6%). Administration of PHA-P increased the percentage of apoptotic $CD4^+$ cells on 14 dpi in the mesenteric lymph nodes (30,2%), 21 dpi in the spleen (5,8%) and on 35 dpi in the muscular inflammatory infiltration (5,9%). The apoptosis of $CD8^+$ cells was stimulated in the spleen on 21 and 28 dpi (1,2 and 4,5%) and on 14 and in the mesenteric lymph nodes (13,4%). Percentage of apoptotic $CD8^+$ cells in the muscular inflammatory infiltration was lower over the period of the experiment. Moreover, the number of muscular larvae in PHA-P treated mice was lower than in the control group.

Supported by Ministry of Science and Higher Education, No.2 PO6K 01727

Dr. Jolanta Piekarska
Wroclaw University of Environmental and Life Sciences
Department of Internal and Parasitic Diseases, Faculty of Veterinary Medicine
Address: Pl. Grunwaldzki 47
50-366 Wroclaw
Poland
Phone: 48713205420
e-mail: jola@ozi.ar.wroc.pl

19. The effect of bile on protein excretion/secretion by L1 larvae of *Trichinella spiralis* and *Trichinella britovi*

Dea-Ayuela María Auxilidadora, F. Bolas Fernández

Universidad CEU-Cardenal Herrera, Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Edificio Seminario S/N, 46113- Moncada, Valencia, Spain

Previous in vitro studies (Man Warren et al., 1997, Infection and Immunity, 65, 4806-12) have demonstrated that penetration of *Trichinella* L1 larvae into intestinal cell lines following digestion of the muscle infected carcasses requires previous exposure to small intestine or to its contents. Thus activation can be achieved by incubation of larvae with intestinal contents or bile. When the larvae are incubated with bile the most noticeable effects are a substantial increase in larval motility and protein secretion to the culture medium. In order to look in detail at these proteins and the role they play during intestinal invasion a proteomic approach was undertaken. L1 larvae of *T. spiralis* and *T. britovi* released after artificial digestion from mouse carcasses experimentally infected were incubated with bile from dog, cow and pig used at 1:20 dilution in RPMI-1640 for 3h at 37şC and 5% CO_2. Following this incubation the media were replaced by fresh RPMI-1640 and allowed to stay for 24 hours under the same culture conditions. Then the media were collected and the proteins were precipitated in methanol-chloroform. The precipitated proteins were submitted to separation by 2- dimensional gel electrophoresis (2DE) and visualised by colloidal coomassie blue staining. Protein maps are being compared to a 2DE reference map (Robinson & Connolly, 2005, Proteomics 5, 4525-32) to be able to identify species-specific variations in protein secretion by L1 larvae against incubation with bile from the 3 different hosts.

Work funded by MedVetNet/Trichimed/WP27

María Auxiliadora Dea-Ayuela, PhD
Universidad CEU-Cardenal Herrera,
Atención Sanitaria
Salud Pública y Sanidad Animal
Facultad de Ciencias Experimentales y de la Salud,
Edificio Seminario S/N
46113-Moncada, Valencia, Spain
Phone: +34 961369000
e-mail: mda_3000@yahoo.es

20. An early antibody response is produced against muscle larva deglycosilated excretory-secretory proteins by *Trichinella spiralis* infected rats

del Rosario Salinas-Tobón, María[1], A. Moreno-Perez[1], Eva Jacinto-Loeza[1], J. Hernández-Sánchez[2]

[1]Escuela Nacional de Ciencias Biológicas, Department of Inmunología, Instituto Politécnico nacional, México
[2]Departamento de Genetica y Biología Molecular, CINVESTAV, IPN. San Pedro Zacatenco, México

Studies about the characterization of NBL antigens and their role in the host-parasite interplay are scarce. Recently, it was shown that *T. spiralis* infected rats produce an early antibody response against a 49 kDa newborn larvae (NBL) antigen and that this may be antigenically related to the 49, 53 and 62 kDa muscle larvae escretory-secretory products (ML-ESP). In this work, we further characterized the 49 kDa NBL protein and analyzed whether it is antigenically related to ML-ESP. We also analyzed its localization inside the NBL. To this aim, a polyclonal antibody specific for the 49 kDa NBL antigen (PoAb) was used to immunodetect NBL total soluble proteins (TSP) separated by two-dimensional electrophoresis, to immunodetect ML-ESP before and after being deglycosilated with trifluoromethanesulfonic acid and to cytolocalize the 49 kDa NBL antigen in the NBL. In addition, the kinetics of antibody production by *T. spiralis* infected rats against deglycosilated ML-ESP and the antigens recognized by these antibodies were studied. The two dimensional analysis of NBL-TSP showed a group of abundant acidic proteins. Immunodetection with PoAb revealed that the 49 kDa NBL antigen is composed of 4 acidic proteins. At least seven Coomassie blue-stained deglycosilated ML-ESP of 30, 34, 36, 40-42, 58, 62 and 74 kDa were observed, among these, the 30, 34 and 37 kDa components were the most abundant. PoAb reacted weakly with 49 and 53 kDa and strongly with 62 kDa proteins from untreated ML-ESP. This antibody recognized the 50, 58, 62, 151, 162, 223 and 234 kDa proteins from deglycosilated ML-ESP outstanding the 62 kDa antigen. Internal structures in the anterior half of the parasite were recognized by the same antibody. *T. spiralis* infected rats produced an early antibody response against deglycosilated ML-ESP at days 12, 14 and 17 post infection, while the response to ML-ES components started from day 17 and increased thereafter until the end of the study. Early antibodies recognized a 40-42 kDa deglycosilated ML-ES protein. Although this protein was not identified by PoAb, a 40-42 kDa component was detected in NBL-ESP and the 49 kDa component

from NBL-TSP in addition to the 50, 58, 62, 151, 162, 223 and 234 kDa components from deglycosilated ML-ESP. Epitopes recognized by PoAb were more abundant in NBL-TSP and deglycosilated ML-ESP than in ML-ESP. Altogether these results, suggest that the early antibody response is primarily directed against the protein core of the secreted proteins and late in the infection to carbohydrate residues due to protein glycosylation during NBL maturation. Thus, proteins released by NBL or ML may be useful for early diagnosis of trichinellosis and to analyze their function in the early events of ML-nurse cell development.

*Fellow of COFAA, EDD

Maria del Rosario Salinas-Tobón, PhD
Escuela Nacional de Ciencias Biológicas
Department of Imunología
Instituto Politécnico nacional
Prol. de Carpio y Plan de Ayala S/N,
México, D.F. 11340 México
Phone: +52 55 57296000 ext. 62486
e-mail: msalinas@ipn.mx

21. Response of galectin-1 deficient mice to *Trichinella* infection, a preliminar study

María J. Perteguer[1], E. Rodríguez[1], J.J. Nogal-Ruiz[1], M.A. Pineda[2], F.M. Ubeira[3], P. Bonay[4], T. Gárate[1]

[1]Departamento de Parasitología. Facultad de Farmacia, UCM, Madrid, Spain
[2]Centro de Biología Molecular, Universidad Autónoma de Madrid, Madrid, 28049, Spain
[3]Laboratorio de Parasitología, Facultad de Farmacia, USC, Santiago de Compostela 15782, Spain
[4]Centro de Biología Molecular, Universidad Autónoma de Madrid, Madrid, 28049, Spai

Galectins are a family of evolutionarily conserved carbohydrate binding proteins. These proteins have recently attracted the attention of immunologists as novel regulators of both inflammation and autoimmunity. *Trichinella* infection produces a chronic disease in which each stage of the life cycle can evoke a host immune response. Thus, expulsion of intestinal parasites has been shown to be T cell dependent. It has been suggested a pivotal role for galectin-1 in the establishment and maintenance of T cell tolerance and homeostasis. In the present work, we investigated the response to *Trichinella* infection in knock out gal 1-/- mice. Wild type and deficient mice were infected with either *Trichinella spiralis* or *Trichinella pseudospiralis* (300 larvae p.o) and the number of muscle larvae was determined 35 days later. In mice infected with *T. spiralis*, the larve recovered was greater in the Galectin-1-deficient mice than in the wild type (2519.4 larvae/g *versus* 1892.8 larvae/g, $p<0,05$). A similar response was observed when mice were infected with *T. pseudospiralis*, (1283.8 larvae/g in gal1-/- versus 1171.2 larvae/g in the wild type, $p<0,05$). *Urban et al*., (2001) using recombinant mice (cytokine deficient mice) found an association between Th2 cytokine and *T. spiralis* host protection. A critical role has been described for galectin-1 in shifting the balance from Th1 toward a Th2-polarized immune response. So, we evaluated the cytokine responses at days 0, 8, 15 and 35 postinfection (p.i.), using a Th1/ Th2 panel of cytokines.

This work was supported by TRICHIMED grant (MPY13/04-27) of the MED-VET-NET project (FOOD-CT-2004 506122) and by the RETIC project (RICET; RD06/0021/0019).

María J. Perteguer
Instituto de Salud Carlos III
Department of Parasitología
Centro Nacional de Microbiología
Ctra. Majadahonda-Pozuelo Km. 2,2
28222 Majadahonda
Madrid/Spain
Phone: +34918223603
e-mail: chus.perteguer@isciii.es

22. Delineation of an immunodominant epitope of *Trichinella* NBL stage

BaoQuan Fu[1], Liu Mingyuan[2], Li Chengyao[2], Wu Xinping[2], P. Boireau[3]

[1]Lanzhou Veterinary Research Institute, CASS, Lanzhou, China
[2]Key Laboratory of Zoonoses,Jilin University, , 130062 Changchun, P. R. China
[3]JRU BIPAR INRA AFSSA ENVA UPVM, LERPAZ, MAISONS-ALFORT cedex, France

Newborn larvae (NBL) stage represent the invasive form of *Trichinella* through its host. Analysis with several monoclonal antibodies revealed the presence of stage specific epitopes on the surface of the NBL. Stage specific genes were selected using suppression subtractive hybridization (SSH) technique and were expressed in E coli to test their antigenicity. 22 clones were identified by SSH. The length of the cloned cDNAs ranged from 305 to 1504 base pairs (bp), with an average length of 731 bp. Half of these clones had no matches in the previously established *T. spiralis* ESTs database, and 4 showed no homology to any known database genes. Two clones were stage-specific with an on/off expression. The first one, NBL1 is a new gene coding for a serine protease which is supposed to be associated with *Trichinella* invasion. The recombinant NS1 is strongly recognised by serum of pig experimentally infected. The epitope mapping of the protein NBL1 was performed and a peptide of 15 amino acid was delineate that allow to mimic the E/S antigen in ELISA test.

This work was supported by grants of EU TRICHIMED (MedVetNet) and grant of China 863 program 2006AA02Z451

Pascal Boireau, PhD
AFSSA, INRA, ENVA, UPVM
JRU BIPAR, AFSSA LERPAZ
AFSSA LERPAZ
23 av du Général de Gaulle
94700 Maisons Alfort
FRANCE
Phone: 33 1 49 77 13 00
e-mail: p.boireau@afssa.fr

23. Influence of intestinal immunity on myositis induced by *Trichinella spiralis*

Fabre, Valeria, D.P. Beiting, Lucille F. Gagliardo, Susan K. Bliss

We previously demonstrated that IL-10 is critical in the control of acute inflammation elicited by *Trichinella spiralis* muscle infection. In this study we investigate muscle inflammation in the context of a natural infection and we define the impact on myositis of immune responses induced by intestinal infection. We use IL-10 and IL-4/10 deficient mice together with quantitative measures of inflammation and cytokine responses. Myositis induced by *T. spiralis* infection was exacerbated by the intestinal immune response. Enhanced inflammation was not associated with dramatic alterations in IL-4, 5, 13 or IFNgamma production by cells in draining lymph nodes, but may relate to reduced IL-10. IL-10 limited the volume of muscle infiltrates. Furthermore, it limited TH2 cytokines, TNFalpha, and NO production by draining lymph nodes cells as well as iNOS expression in macrophages and eosinophils that infiltrated muscle. In the absence of IL-10, the influence of IL-4 was largely on the composition of muscle infiltrates. At one time point, the lack of Th2 responses in IL-4/10 deficient mice resulted in reduction of myositis. In these mice increased production of TNFalpha and NO occurred together with weight loss and morbidity. Thus, intestinal infection with *T. spiralis* induced IL-4 and IL-10 that demonstrate both distinct and cooperative effects in regulating myositis.

Valeria Fabre, Postdoctoral Associate
Cornell University, James A. Baker Institute for Animal Health
Hungerford Hill Rd
14853 Ithaca NY USA
Phone: (001)607-256-5647
e-mail: mvf7@cornell.edu

24. Effect on apoptosis-associated protein expression of H7402 cell co-incubating with *Trichinella spiralis* crude extract

Wang Xuelin[1], Xiuping Wu[1], Xinrui Wang[1], Guozhen Cui[1], Mafeng Liu[1], Ying Zhao[1], Xiangye Liu[1], Yanling Yu[1], Hongkuan Deng[1], Boireau Pascal[2], Mingyuan Liu[1]

[1]Key Laboratory of Zoonoses, Ministry of Education, Jilin University, Institute of Zoonoses, Changchun, P. R. China
[2]JRU BIPAR INRA AFSSA ENVA UPVM, 94703 MAISONS-ALFORT cedex, France

Worldwide,hepatocellularcarcinoma(HCC) is the fifth most common cancer and the third most common cause of cancer-related death, however, with the arrival of newly developed molecularly targeted agents and the success of some of these agents in other traditionally challenging cancers. To look for source of molecularly targeted agents is urgent necessary. It will be renewed interest in developing systemic therapy for HCC. *Trichinella spiralis* (*T. spiralis*) confers resistance to tumor cells and rarely leads to significant morbidity. It presents an interesting and potentially useful approach to therapeutics HCC. *T. spiralis* will be a hopeful source of molecularly targeted agents in therapy of HCC. In vitro, we tested wound repair by wound repair model, cell proliferation by MTT, H7402 invasion, cytoplasmic and intramitochondrial free Ca2+ concentrations, and mitochondrial membrane potential breakdown by confocal microscope. Effect of *T. spiralis* crude extract to close the wound as soon as 8 hours after wounding, crude extract showed reducing wound closure over control. The crude extract significant decrease in cell number was seen in the cells treated with contain total protein 0.035, 0.070 and 0.140mg/ml on H7402 for 24h. H7402 treated with *T. spiralis* crude extract for 8 hours showing that virally dramatically blocked cell invasive migration. *T. spiralis* crude extract effect to close the wound, antiproliferration, antiinvasion possible reason is H7402 cell apoptosis induced by *T. spiralis* crude extract. Work founded by China 863 program 2006AA02Z451

Xuelin Wang, PhD
Key Laboratory of Zoonoses, Ministry of Education, Jilin University, Institute of Zoonoses
Xian Road 5333
130062 Changchun P. R. China
Phone: +8643187836713
e-mail: wxlwqx@sohu.com
Web: http://www.jluhp.edu.cn/zuzhijigou/yanjiusuo/index.htm

25. Stimulation of pig mast cells by *Trichinella* antigens triggers Th2 cytokine mRNA expression

Isabelle Vallée[1], Mélanie Picherot[1], Véronique Lainé-Prade[1], P. Boireau[2]

[1]AFSSA-LERPAZ, Department of UMR BIPAR, 23 avenue du Général de Gaulle, 94706 France
[2]UMR BIPAR, AFSSA LERPAZ, 23 avenue du Général de Gaulle, 94706 Maisons-Alfort, France

Mice mast cells are known to release histamine and IL-4 after few minutes with *Trichinella spiralis* antigens stimulation. These two mediators are both described to induce orientation of dendritic cells (DC) towards a DC2 pattern. In order to understand the role of mast cells in the induction of the protective immune response against Trichinella, we focused our study on pig mast cells and their activation by *T. spiralis* antigens. Cells were stimulated by two antigens from *T. spiralis*: TSL1 and NBL1. TSL1 induced a stronger cellular degranulation than NBL1 after 15 minutes of stimulation. Regarding cytokines mRNA expression, IL-4 was not significantly modulated but IL-13 was strongly induced by TSL1 and more moderately by NBL1. IL-5, IL-10 and TNF-a mRNA were also induced by the two antigens. Regarding surface markers, TSL1 induced FceRI, TLR-4, CD58 and CD86 whereas NBL1 modulated only CD58. Trichinella antigens did not increase CD28 mRNA. In conclusion, Trichinella antigens activated pig mast cells, but differences were observed according to the antigens used, suggesting stimulation of different pathways. Mast cells were induced to produce cytokines mRNA related to a Th2 profile as well as costimulatory molecules. These results suggest that during Trichinella invasion, mast cells could produce a cytokine micro-environment in favour of a DC2 differentiation, and could also be involved in interactions with local immune cells. Supported by AFSSA-INRA "ImmunoMucoPorc".

Coresponding autor:
Isabelle Vallée, Dr
AFSSA-LERPAZ
Department of UMR BIPAR
23 avenue du Général de Gaulle
94706 France
Phone: 33 1 49 77 28 16
e-mail: ivallee@vet-alfort.fr

26. Differential activation of mast cells by antigens from *Trichinella spiralis* muscle larvae, adults and new born larvae

Yépez-Mulia, Lilian[1], Claudia Montaño-Escalona[1,2], R. Fonseca-Liñán[2], Narcy Arizmendy-Puga[1], P. Boireau[3], Guadalupe Ortega-Pierres[2]

[1]UIMEIP-Pediatria, IMSS. Mexico
[2]Department of Genetics and Molecular Biology, Cinvestav-IPN. Mexico
[3]UMR BIPAR, Maisons-Alfort, France

Mast cells (MC) hyperplasia and activation are prominent features in *T. spiralis* infection. Indeed it has been shown a temporal correlation between the kinetics of intestinal mastocytosis, release of inflammatory mediators from MC and adult worm loss a major component of the defense against *T. spiralis* infection. It is well known that during the intestinal phase of *T. spiralis* infection, muscle larvae (ML) and adult worms (AD) enter into contact with the host, however interaction with MC may occur during migration of new born larvae (NBL). Therefore, it is plausible that antigens from these developmental stages could activate MC. We have previously demonstrated by in vitro assays that TSL-1 antigens activate MC through an Ig-independent mechanism leading to the release of histamine, MC protease 5, IL-4 and TNF alfa. In this work we evaluated if total antigens from AD or from NBL could activate unsensitized MC and we compared this activation with the one detected when MC are stimulated with TLS-1 antigens. MC activation was also tested with affinity chromatography purified antigens from NBL using the monoclonal antibody CE-4 that recognizes NBL surface components. The results obtained in this study showed that total adult extracts induced the release of histamine but no beta hexosaminidase from unsensitized MC. Interestingly, similar results were obtained when MC were activated with TSL-1 antigens, suggesting a selective secretion of MC mediators. In contrast total NBL extracts or purified NBL antigens did not induced the release of either histamine or beta hexosaminidase from MC. All together these results suggest that antigens from ML and AD induce a differential activation of MC in vitro. Interestingly, these two stages are those that interact with the host during the intestinal phase of infection. The mechanisms involved in TSL-1 and AD activation of unsensitized MC may function together with other mechanisms of MC activation in host protection against *T. spiralis*. We are currently purifying adult antigens in order to identify the specific antigens involve in MC activation.

27. Attempts to infect in vitro mammalian myotubes with *T. spiralis* newborn or muscle larvae

Valentin – Tirado, Griselle, W.J. Kozek

Department of Microbiology and Medical Zoology, Medical Sciences Campus, University of Puerto Rico

Previous reports that mature *T. spiralis* muscle larvae can infect and develop nurse cell complexes in vivo prompted us to determine whether cultured mammalian myoblasts could also be infected, with resulting nurse cells, either by newborn or muscle larvae, and to repeat the in vivo studies of nurse cell formation after subcutaneous or intramuscular inoculation of muscle larvae. ATCC L6 cell line, originally obtained from primary cultures of rat thigh muscles, was purchased from ATCC and established in our laboratory in Dulbecco's Modified Eagle's Medium containing L-glutamine, sodium bicarbonate, glucose, sodium pyruvate and 10% fetal calf serum. Newborn larvae were obtained from culture of adult worms that were recovered from intestine of infected rats at 4th day after inoculation; muscle larvae were obtained from muscle digests of rats infected four weeks previously. After ATCC L6 cells were allowed to mature in culture until contractile elements and contractions could be discernible in many myotubes, the cultures were inoculated with either newborn larvae, or muscle larvae, and monitored by examining all cultures by light microscopy. Neither the newborn or muscle larvae penetrated the myotubes in their respective cultures during the period of observation (9 days for newborns, 15 days for muscle larvae. No obvious changes in the myotubes were observed in cultures inoculated with newborn larvae; however, cultures inoculated with muscle larvae contained muotubes which presented agglomeration of nuclei, bulging and vacuolation as early as 24 hours after addition of larvae. Subcutaneous or intramuscular inoculation of muscle larvae apparently did not produce formation of nurse cells; the nurse cells detected 15 days after inoculation can be attributed to oral infection by animals that have cleaned themselves, or their cage-mates, after injections. Our results support previous unsuccessful attempts to infect cells in vitro, and suggest that the ability of muscle larvae to infect cells be further investigated.

Supported, in part, by the Deanship of Biomedical Sciences, Medical Sciences Campus, UPR, RCMI Award G12RR-03051 from the NCRR, NIH, USA, and the MBRS Program.

Wieslaw J. Kozek
Department of Microbiology and Medical Zoology
Medical Sciences Campus
University of Puerto Rico
P.O. Box 365067
San Juan, PR 00936-5067
Tel. 1-787-758-2525, ext. 1351
e-mail: wkozek@rcm.upr.edu
Fax: 1-787-758-4808

28. Inflammatory response to different *Trichinella* species evaluated by an image analysis method

Bruschi, F.[1], A. Casulli[2], E. Pozio[2], M. Masetti[3]

[1]Department of Experimental Pathology, M.B.I.E., Universita' di Pisa
[2]Istituto Superiore di Sanita, Rome, Italy
[3]Dipartimento di Biologia, Universita di Pisa, Pisa, Italy

The aim of this study was to evaluate the inflammatory response and its effects on nematode parasites of the genus Trichinella which induce (*Trichinella spiralis* and *T. britovi*) or not (*T. pseudospiralis, T. papuae, T. zimbabwensis*) the development of a collagen capsule around the muscle cell-parasite complex in the host muscles.
Materials and methods: a method was set up to estimate the inflammatory response in histological sections from muscle tissues of *Trichinella* CD1 infected mice, staining the nuclei of muscle cells with a fluorescent probe which binds to nucleic acids, and analysing the fluorescence with an appropriate software. The increase of nucleus number was considered an inflammation marker, since non-infected muscles have a low nucleus density. An evaluation of the relative fluorescence units was performed in both peri-capsular (close to the nurse cell-parasite complex) and extracapsular (where the parasites is not present) areas. Muscle larvae from both encapsulated and not encapsulated species were tested with a polyclonal antibody anti-nitro tyrosine by immunoblot, to evaluate possible differences in the nitrosylation pattern.
Results: inflammation induced by T. spiralis is statistically higher than that elicited by the other *Trichinella* species, both in peri- and extracapsular areas. Encapsulated species show a more evident nitrosylation pattern than the non-encapsulated ones.
Conclusion: the evaluation method of inflammation, set up and tested in the present study, helps to highlight differences of the host response to different Trichinella species in the muscles.

Fabrizio Bruschi, Professor
Universita' di Pisa
Department of Experimental Pathology, M.B.I.E.
Via Roma 55
56126 Pisa Italy
Phone: +39(050)2218547
e-mail: fbruschi@med.unipi.it

29. Complement membrane attack complex formation and infectivity of *Trichinella spiralis* and *T. nativa* in rats

Näreaho Anu[1], S. Saari[1], S. Meri[2], A. Sukura[1]

[1]Department of Basic Veterinary Sciences, University of Helsinki
[2]Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Finland

Rats readily become infected with *Trichinella spiralis* but are more resistant to *T. nativa*. We infected complement factor C6-deficient (C6-) rats and control (C6+) rats with *T. spiralis* and *T. nativa* to compare the effects of membrane attack complex on these parasites in vivo. The 2000 larvae infection dose per rat (equivalent to 7-8 larvae per gram, lpg) yielded 652 lpg in the C6- group and 608 lpg in the C6+ group with *T. spiralis*, whereas with *T. nativa* the corresponding figures were only 1.05 and 1.87 lpg. The difference between the *Trichinella* species was evident, but the infection intensity was unaffected by the C6 deficiency. When newborn larvae were incubated in C6-deficient and control rat sera for 24h in vitro, no changes in viability were observed. Immunohistochemistry revealed that the musculature of cross-sectioned adults and certain stichocytes bound human complement factors C3, C8 and C9, but not C1q. Interestingly, the outermost layer of the cuticle and the newborn larvae did not show any binding activity. Similar findings were obtained with immunofluorescence microscopy of intact newborn larvae. These results indicate that both *T. spiralis* and *T. nativa* have efficient mechanisms to protect themselves against complement attack. The difference in infectivity for rats between the two species, however, is not due to a differential resistance to complement membrane attack complex.

Anu Näreaho, PhD.
University of Helsinki
Department of Basic Veterinary Sciences
Faculty of Veterinary Medicine
P. O. Box 66, 00014 Helsinki Finland
Phone: +486 62 58 67 00
e-mail: anu.nareaho@fimnet.fi

30. Changes in murine cellular immunity after *Trichinella spiralis* infection with low doses

Dvoroznaková, Emília[1], Zuzana Hurníková[1], Marta Kolodziej-Sobocińska[2], Ewa Dziemian[2]

[1]Parasitological Institute SAS, Košice, Slovak Republic
[2]Institute of Parasitology PAS, Warsaw, Poland

The low infective doses of *Trichinella spiralis* simulated natural conditions of the infection in rodents, important reservoirs of trichinellosis. The regulative and effector components of immunity (T lymphocytes, macrophages) and their functional activity were studied. Mice BALB/c were infected with 5 larvae of *T. spiralis* and selected immunological parametres were examined within 20 days post infection (p.i.). The low infection induced a significant increase in the proliferative activity of both T and B cells for a short time on day 15 p.i. The percentage of CD4 and CD8 T cell subpopulation in the spleen of infected mice did not show any important changes in comparison to control. However, the absolute numbers of splenic CD4 and CD8 T cells were decreased on day 5 p.i. and 20 p.i. Peritoneal macrophages of infected mice increased their production of superoxide anion from day 15 p.i., with the peak on day 20 p.i. The numbers of T. spiralis adults in the small intestine of mice were decreasing from day 5 (2.67ą0.58) to 15 p.i. (1.33ą0.58) per mouse. On day 20 p.i. adults were eliminated and muscle larvae of infected mice were obtained in numbers of 71.33ą10.60 per mouse. Low doses of *T. spiralis* larvae also induce development of the cellular immune response of infected mice, particularly during migration of newborn larvae, on 2nd week p.i.

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-51-010704 and Slovak Research and Development Agency under the contract No. APVV-51-027605.

Emília Dvoroznakova, DVM
Parasitological Institute SAS
Hlinkova 3
040 01 Kosice, Slovak Republic
Phone: ++421 55 6334455
e-mail: dvoroz@saske.sk

31. *Trichinella spiralis* infection and autoimmunity. II. Chronic parasite infection has beneficial effects on the course of experimental autoimmune encephalomyelitis

Gruden-Movsesijan, A.[1], Lj. Sofronić-Milosavljević[1], N. Ilić[1], M. Milić[1], S. Stošić-Grujičić[2], M. Mostarica-Stojković[3]

[1]Institute for the Application of Nuclear Energy - INEP, University of Belgrade
[2]Department of Immunology, Institute for Biological Research "Siniša Stanković", University of Belgrade
[3]Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Serbia

The established model, in which chronic *Trichinella spiralis* (*T. spiralis*) infection ameliorates the course of experimental autoimmune encephalomyelitis (EAE) in Dark Agouti (DA) rats, was used to investigate helminth-induced regulatory mechanisms that contribute to beneficial effects to the host. DA rats were infected with 500 *T. spiralis* muscle larvae/animal and after 28 days immunized with syngeneic spinal cord homogenate in Complete Freunds adjuvant. Several experiment runs confirmed that significant suppression of clinical manifestations of EAE was achieved (as judged by decreased clinical scores and CNS cellular infiltrates). Cytokine profiles of *T. spiralis* infected and non-infected DA rats before and 8 days after EAE induction were analysed by measuring lymph node cell cytokine production, both spontaneous and induced by nonspecific and parasite-specific in vitro stimulation. Increased levels of Th2 cytokines (IL-4 and IL-10) and almost unchanged level of IFN as a result of *T. spiralis* infection were found compared to controls. The Th2 cytokine bias was maintained even when EAE was induced in rats infected with *T. spiralis*. Adoptive transfer of spleen mononuclear cells from *T. spiralis* infected animals to animals 3 days after EAE induction, significantly reduced the incidence and severity. Both observed phenomena, *T. spiralis* induced production of anti-inflammatory cytokines such as IL-10 and demonstrated ability of infection-sensitized cells to transfer protective effects against EAE, could indicate that helminth orchestrated regulatory cells are involved. We suggest that the beneficial effect of *T. spiralis* infection on EAE is accomplished by established Th2 cytokine milieu and by regulatory mechanisms yet to be defined. (Grants No: 143047, 143029, 145066)

Alisa Gruden-Movsesijan, PhD
Institute for the application of nuclear energy - INEP
Department for immunology and immunoparasitology
Banatska 31 b
11080 Belgrade Serbia
Phone: 381 11 619 525
e-mail: alisa @inep.co.yu

32. Characterization of rat bone marrow dendritic cells initially primed by *Trichinella spiralis* antigens

Ilić, N.[1], M. Čolić[2], A. Gruden-Movsesijan[1], I. Majstorović[2], S. Vasilev[1], Lj. Sofronić-Milosavljević[1]

[1]Institute for the Application of Nuclear Energy - INEP, University of Belgrade, Belgrade
[2]Institute for medical research, Military Academy, Belgrade, Serbia

Pathogen-derived products have the capacity to induce maturation of bone marrow-derived dendritic cells (BMDCs) into effector cell populations that polarize Th cells toward Th1 or Th2 phenotype via different mechanisms. Since those mechanisms are not clarified for helmints yet, we investigated the impact of *T. spiralis* (TS) antigens (5 different antigen isolates from all 3 life-cycle stages of parasite) on maturation of BMDCs and their potential to present TS antigens to infection-sensitized T cells. The expression of MHC class II, co-stimulatory molecules CD 86 and CD 54 as well as IL-10 and IL-12p70 cytokine production were measured after 2 days of BMDCs cultivation with TS antigens. While parasitic antigens did not significantly alter the expression of MHC II, most of them, except muscle larvae crude antigen, up-regulated the expression of costimulatory molecules. All applied antigens increased and decresed the production of IL-10 and IL-12p70, respectively, by BMDCs. Significant proliferation of singenic cells isolated from lymph nodes of TS infected DA rats was induced by BMDCs pulsed with each applied antigen. Muscle larvae antigens, either metabolic or ConA fractionized, had the most profound effects. BMDCs, primed with TS antigens, up-regulated the production of IL-4 by T cells purified from lymph nodes of infected singenic rats. The most prominent effect was seen using BMDCs stimulated with metabolic products of adults or muscle larvae. Obtained results indicate that stimulation of BMDCs with TS antigens leads to the polarization of immune response towards Th2 type.
(Grant No 143047)

Natasa Ilic, MSc
Institute for the application of nuclear energy - INEP
Department for immunology and immunoparasitology
Banatska 31 b
11080 Belgrade Serbia
Phone: 381 11 619 525
e-mail: natasa@inep.co.yu

33. *T. spiralis* infection and autoimmunity. I. The establishment of model system in DA rats

Sofronić-Milosavljević, Lj.[1], A. Gruden-Movsesijan[1], M. Milić[1], N. Ilić[1], S. Stošić-Grujičić[2], M. Mostarica-Stojković[3]

[1]Institute for the Application of Nuclear Energy - INEP, University of Belgrade,
[2]Department of Immunology, Institute for Biological Research "Siniša Stanković", University of Belgrade,
[3]Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Serbia

Helminth infections have been recognized as potent modulators of immune response in experimentally induced autoimmune diseases, but underlying mechanisms are not resolved yet. Capacity of chronic *Trichinella spiralis* infection to modulate immune response by inducing Th2 dominated cytokine profile and provoking anti-inflammatory and immunosupressive mechanisms in the host, propose this parasite for such investigations. The aim of this study was to evaluate an influence of *T. spiralis* infection on the development and course of experimental autoimmune encephalomyelitis (EAE) in Dark Agouti (DA) rats. Rats were infected orally with different doses of infective muscle larvae (L1) and were allowed to recover until day 28 post infection, before the induction of EAE with syngeneic spinal cord homogenate in Complete Freund's adjuvant. Intermediate doses of *T. spiralis* infection tested (500, 1000, 2000 L1) caused significant decrease in mean maximal severity score of EAE clinical signs compared to uninfected EAE animals (controls) (0.8 ą 1.1 vs. control 3 ą 0.7; 2.2 ą 0.8 and 2.3 ą 0.8 both vs. control 3 ą 0.3, respectively). Histopathological changes in spinal cord tissue of animals with concomitant *T. spiralis* infection and EAE demonstrated reduction of mononuclear cell infiltrates. Low and high doses (100 and 5000 L1) infected animals developed similar clinical signs as controls. Results of our experiments demonstrated that *T. spiralis* infection led to autoimmune disease amelioration. This phenomenon was infection dose dependent. (Grants No: 143047, 143029, 145066)

Ljiljana Sofronic-Milosavljevic, PhD.
Institute for the application of nuclear energy - INEP
Department for immunology and immunoparasitology
Banatska 31 b
11080 Belgrade Serbia
Phone: 381 11 619 525
e-mail: sofronic@inep.co.yu

34. Investigation on the efficacy and immunologic mechanism of *Trichinella spiralis* intervening experimental inflammatory bowel disease

Ying Zhao, Xuelin Wang, Xiuping Wu, Xinrui Wang, Hong Bin Zou, Hongkuan Deng, Lei Sun, Mingyuan Liu

Based on the assumption that generation of a Th2 response by nematode *Trichinella spiralis* (*T. spiralis*) could prevent or reduce the effects of Th1-mediated inflammatory bowel disease (IBD), the possibility that *T. spiralis* as a kind of immunomodulator prevent and cure IBD by adjusting colonic immunity will be investigated in this study. The colitis mice, experimentally established by trinitrobenzesulfonic acid (TNBS) and oxazolone (OXZ) respectively, were infected with T. spiralis, the effect for mice colitis prevention and treatment were evaluated by index checking of DAI scores, histological damage, index of inflammation, the transcription and expression of Th cytokines on molecule lever, protein lever, colonic immunity, general immunity, and the immunologic mechanism was also investigated. *T. spiralis* infection significantly attenuates TNBS-induced colitis in the mice. The immunologic mechanism is that *T. spiralis* can down-regulate strong Th1-type immune response of colitis and up-regulate Th2 response. Prior *T. spiralis* infection doesn't reduce the severity of OXZ-induced colitis. Proper doses of IL-10 can ameliorate inflammation, but high doses of IL-10 not only decrease the ability of regulating excessive dysregulated Th2 responses but also induce much proinflammatory cytokine in OXZ-induced colitis. The above indicates that if we can find the cause of inducing high doses of IL-10 and change it, T. spiralis will likely induce proper doses of IL-10 to result in efficacy on Th2 responses. The study offers a valuable theory and practice evidence for further research that hurtless *T. spiralis* our owning or purified effective antigen prevent and cure IBD, and consequently provide a kind of safe and effecive immunomodulator for IBD. Work founded by National Natural Science Foundation of China (NSFC30600276) and China 863 program 2006AA02Z451.

Ying Zhao, PhD
Key Laboratory of Zoonoses, Ministry of Education,
Jilin University, Institute of Zoonoses
Xian Road 5333
130062 Changchun P. R. China
Phone: +8643187836713
e-mail: wxlwqx@sohu.com
Web: http://www.jluhp.edu.cn/zuzhijigou/yanjiusuo/index.htm

35. Effect of albendazol on the antibody response and muscle larvae establishment of *Trichinella spiralis* infected rats

del Rosario Salinas-Tobón, María[1], A. Moreno-Perez[1], Fabiola Velasco-Rivera[1], R.A. Avendańo-Rabiella[1], J. Hernández-Sánchez[2]

[1]Escuela Nacional de Ciencias Biológicas, Department of Inmunología, Instituto Politécnico nacional, México
[2]Departamento de Genetica y Biología Molecular, CINVESTAV, IPN. San Pedro Zacatenco, México

Early and late antibody responses to *Trichinella spiralis* have been analyzed in several host species. Antibody detection at the beginning of the infection is essential for an appropriate anthelmintic treatment. However, systematic studies to assess the trichinellosis treatment efficacy with albendazol using an appropriate serological method are insufficient. Thus, we analyzed the effect of albendazol (ABZ) during the early larva encapsulation phase on the whole antibody (Ab) response and on muscle larvae (ML) establishment and infectivity. Four-rat groups were infected with 2000 *T. spiralis* ML. Rats were treated with vehicle (B) or ABZ, 20 mg/Kg/day for 3 (C) or 6 days (D) starting on day 13 post-infection (pi). Serum samples collected before and at different times after the infection were analyzed by ELISA using newborn larvae total soluble protein (NBL-TSP), ML excretory-secretory products (ML-ESP), deglycosilated ML-ESP (ML-dESP) or ML total soluble protein (ML-TSP). ML recovery was done at day 61 pi by digesting artificially the gastrocnemius to determine ML establishment and viability. Infectivity of recovered ML was tested in mice. The Ab response against NBL-TSP and ML-TSP appeared on day 10 and to ML-ESP on day 17 pi in untreated rats. An early production of Abs to NBL-TSP, ML-TSP and ML-dESP peaked on day 14 pi. The highest antibody production to NBL-TSP was on day 14 and to ML-TSP, ML-ESP and ML-dESP antigens on day 61. Group B showed similar results as group A. In group C, as compared with groups A and B, a transitory decrease of the Ab response to NBL-TSP and ML-dESP antigens was observed from days 17 to 26 and to ML-ESP antigens from days 21 to 26, whilst Ab production to ML-TSP was not affected. In group D, reduced Ab levels against NBL-TSP and ML-ESP were more evident. ML establishment in treated rats and viability of recovered ML from group C were not affected, however, infectivity was strongly affected; only 1% of the ML recovered, established the infection in the mouse. ML establishment was also affected by the 6-day ABZ treatment. Several *T. spiralis*

antigens recognized early in the infection included components of 43 and 85 kDa from ML-TSP, of 49 and 205 kDa from NBL-TSP and 40 kDa from ML-dESP while detected late recognition antigens included: 43, 45, 48, 60, 64 and 97 kDa from ML-TSP; 43, 49, 51, 57, 62, 69 76, 84 151, 162, 223 and 234 kDa from ML-ESP; 43, 49, 51, 57, 109, 151, 152, 223 and 234 kDa from ML-dESP. Except for ML-TSP, ABZ treatment diminished the intensity of early recognized components and delayed the detection of late recognition proteins. Overall, these results suggest that not yet defined antigens early recognized in NBL-TSP, ML-TSP and ML-dESP preparations are candidates for the early diagnosis of trichinellosis while those lately recognized in ML-ESP and ML-dESP antigens may be useful to evaluate ABZ treatment efficacy.

*Fellow of COFAA, EDD

Maria del Rosario Salinas-Tobón, PhD
Escuela Nacional de Ciencias Biológicas
Department of Imunología
Instituto Politécnico nacional
Prol. de Carpio y Plan de Ayala S/N,
México, D.F. 11340, México
Phone: +52 55 57296000 ext. 62486
e-mail: msalinas@ipn.mx

36. Immunochromatographic strip for detection of anti-*Trichinella* antibodies in muscle juice of experimentally infected domestic pigs

Cui J., Z.Q. Wang, J. Wang, Y.X. Qin

In order to observe the effect of the immunochromatographic strip for detection of anti-*Trichinella* antibodies in muscle juice of experimentally infected domestic pigs, thirty male Ningxiang pigs were randomly divided into 2 groups: experimental group with 20 pigs and control group with 10 pigs. The experimental group was orally inoculated with 5.000 motile muscle larvae of *T. spiralis*. The anti-*Trichinella* antibodies in serum and muscle juice from the infected and control pigs were assayed by the strip 10 weeks after infection. The results of strip were compared with those of trichinelloscopy. Anti-*Trichinella* antibody positive rate of serum and muscle juice of the infected pigs was 100%(20/20), the larvae were detected in 100% (20/20) of leg muscle samples by trichinelloscopy with 111-400 larvae per gram (lpg) (mean 231.36). The muscle juice and diaphragm samples of normal pigs were examined by the strip and trichinelloscopy, respectively, the results were negative by both methods. The antibody positive rate of the infected pork conserved at -20°C for 8 months was 100%(20/20). The immunochromatographic strip for detection of anti-*Trichinella* antibodies in muscle juice could be used to the preliminary screening for *Trichinella* inspection of fresh and frozen pork.

The work was supported by the Henan Innovation Project for University Prominent Research Talents (2004KYCX013).

J. Cui, Professor
Medical College, Zhengzhou University
Department of Parasitology
Daxue Road 40
Post Code: 450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: cuij@zzu.edu.cn

37. Detection of anti-*Trichinella* antibodies in muscle juice of experimentally infected mice by Immunochromatographic strip

Cui, J., Y.X. Qin, Z.Q. Wang

To establish one rapid immunological method for detection of *Trichinella* in meat, the antibodies in serum and muscle juice from mice infected with T1 were assayed by the immunochromatographic strip at different time after infection. The results of strip were compared with those of ELISA, trichinelloscopy and digestion method. Anti-*Trichinella* antibodies in muscle juice of mice infected with 100,300 and 500 larvae were detected two weeks post-infection (wpi), with antibody positive rate of 50%, 57.1% and 62.5%, respectively. In three groups of mice, the antibody positive rate of muscle juice was up to 100% six wpi. The positive rate of muscle juice was not different with those of serum sample. The positive rate of muscle juice assayed by strip and diaphragm examined by trichinelloscopy was 98.6% in three group of mice 5~7 wpi. Out of 6 samples containing ＜33 larvae per gram (lpg) muscle by digestion method, 5 samples were positive by strip. The antibodies in muscles containing 6 lpg could be detected by the strip. The antibody positive rate of the infected mice muscles conserved at 4 °C for 1~7d and at -20 °C for 1~7 months was 100%. The strip could be used for detection of anti-Trichinella antibodies in muscle juice from fresh, cool and frozen meat. The word was supported by the National Natural Science Foundation of China (No.30471450) and Henan Innovation Project for University Prominent Research Talents (2004KYCX013).

J Cui, Professor
Medical College, Zhengzhou University
Department of Parasitology
Daxue Road 40,
Post Code: 450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: cuij@zzu.edu.cn

38. Anti-*Trichinella* antibody levels in muscle juice of the mice experimentally infected with *Trichinella* spp.

Z.Q. Wang, J. Wang, J. Cui, L.H. Lai, Y.X. Qin

To observe the specific antibody levels in muscle juice of the mice experimentally infected with *Trichinella* spp. and their correlation with serum antibodies, two hundred Kunming mice were randomly divided into 4 groups (50 mice per group), each mouse was inoculated with 300 muscle larvae of T2, T3,T4 or T7 respectively. The antibodies in serum and muscle juice taken weekly from 2 to 6 weeks post-infection (wpi) were detected by ELISA using *T. spiralis* muscle larval ES antigens. Anti-*Trichinella* antibody level in muscle juice of the mice infected with T2, T3, T4 or T7 are similar, the specific antibodies were detected 3 wpi in all of the infected mice with antibody positive rate of 60%, 30%, 30% and 30%,respectively. The antibody positive rate increased to 100% 4 wpi in all infected mice. The antibody level in muscle juice showed significant positive correlation with serum antibodies 2-6 wpi in four groups. The antibody level in all infected muscle samples conserved at 4°C for 7d was not different with that conserved at 4 °C for 1d. The anti-*Trichinella* antibodies were detected in all infected mouse muscles conserved at -20°C for 4 months. The results showed the detection of anti-*Trichinella* antibodies in muscle juice could be used to the preliminary screening of the inspection of fresh, cool and frozen meat with *Trichinella* spp. infection. The work was supported by the National Natural Science Foundation of China (No.30471450).

Z.Q. Wang, Professor
Medical College, Zhengzhou University
Department of Parasitology
450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: wangzq@zzu.edu.cn

39. The diaphragm larval burden and antibody levels in sera and muscle juice of mice infected with *Trichinella* spp.

Cui, J., L.H. Lai, Z.Q. Wang

Thirty-two male Kunming mice were divided into 4 groups, each mouse was inoculated with 50 (A group), 100 (B group), 300 (C group), and 500 (D group) T1 muscle larvae, respectively. The additional 50 mice were divided into 5 groups, each group were inoculated with 500 muscle larvae of T1, T2, T3, T4 and T7, respectively. All mice were slaughtered at 42 days post-inoculation, the larval burdens of diaphragm were observed and the antibodies in sera and muscle juice were detected by ELISA using T1 larval ES antigens. The results showed there was no correlation between the larval burdens of diaphragm and anti-Trichinella antibody levels in sera and muscle juice of A, B, C, D groups of the infected mice, but the larval burdens of diaphragm showed the positive correlation with the infecting dose, and the antibody levels in sera also showed significant positive correlation with those in muscle juice in all four group of mice. There was no correlation between the larval burdens of diaphragm and anti-Trichinella antibody levels in sera and muscle juice of mice infected with five Trichinella species, but there was positive correlation between antibody levels in sera and muscle juice. T1 ES antigens could be used to the detection of anti-*Trichinella* antibodies in sera and muscle juice of the mice infected with other four species of Trichinella (T2,T3,T4 and T7) .The work was supported by the he National Natural Science Foundation of China (No.30471450).

J. Cui, Professor
Medical College, Zhengzhou University
Department of Parasitology
Daxue Road 40
Post Code: 450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: cuij@zzu.edu.cn

40.

Susceptibility of DA and AO rats to *T. spiralis* infection

Vasilev, S., Alisa Gruden-Movsesijan, Nataša Ilić, Ljiljana Sofronić-Milosavljević

Institute for the Application of Nuclear Energy - INEP, University of Belgrade, Belgrade, Serbia

Genetic influences upon host variation in susceptibility to primary *Trichinella spiralis* (*T. spiralis*) infection were not clarified for rats yet. The aim of this study was to investigate susceptibility to infection by *T. spiralis* in Albino Oxford (AO) and Dark Agouti (DA) rats. Two groups consisting of 16 animals per each strain (females, 12 weeks old), were used. Eight DA and eight AO rats were infected per os with 500 *T. spiralis* (ISS 161) infective larvae, the other animals were used as controls. After 30 days, all animals were sacrificed. Muscle larval burden was determined by artificial digestion method. Blood samples as well as lymphocytes from cervical lymph nodes were collected. Lymphocytes were cultivated in vitro in medium alone, or stimulated with T. spiralis muscle larval homogenate and mitogen Concanavalin A (Con-A). Levels of IL-4, IL-10 and IFN-γ were measured in culture supernatants and in sera samples, by ELISA test. Clear strain dependent variations were observed in the number of *T. spiralis* larvae per gram of muscle tissue - for DA (626.7 ± 171) rats the obtained values exceeded many times those found in AO rats (49.8 ± 25.9). Differences, between the strains, were also noticed in cytokine production levels. Finally, our results provide evidence that DA rats have higher susceptibility to *T. spiralis* infection compared to AO strain of rats.

Grant No 143047

Saša Vasilev
Institute for the application of nuclear energy - INEP
Banatska 31 b
11080 Belgrade Serbia

41.

Mouse Experimental Model Of Brain *Trichinellosis*

Ombolo, C.[1], Evelyne Souil[1], Agathe Lebuisson[2], J. Dupoy-Camet[2]

[1]Institut Cochin, U 567 Paris Descartes University
[2]PARIS DESCARTES UNIVERSITY, TRICHINELLA NATIONAL REFERENCE CENTER HOPITAL COCHIN APHP, 27 Fbrg St Jacques, Paris, France

Cerebral complications are a common feature during human trichinellosis. Very few data exist on the physiopathology of brain trichinellosis. The aim of our study was to check if brain lesions were observed in an experimental mice model of trichinellosis. Fifteen mice were inoculated by various doses (400, 800 and 1200) of muscular larvae of *Trichinella spiralis* (ISS 104) Mice were killed at 8, 14, 21, 28 & 35 days post infection (dpi). Intestine were examined for adults and whole carcasses digested. Brains were isolated and deep-frozen in isopentane at -20°C Serial sagittal sections(10 l'm) were prepared using a Leica cryostat (Nussloch, Germany) and stained by HES and Klüver-Barrera. Blood samples were examined for IgG antibodies using an ELISA assay (*Trichinella* kit Biotrin, Lyons, France). Adults were seen in all mice from 8 to 35 dpi. Muscular larvae (147 to 751 larvae per g) were seen in all mice from 21 dpi. Antibodies were detected in mice from 14 dpi. Hemorrhagic brain lesions were observed microscopically in 8 mice : 3/3 mice sacrificed at 8dpi; 3/3 mice sacrificed at 14dpi; 1/3 mice sacrificed at 21dpi & 1/3 mice sacrificed at 35 dpi. Interestingly, mice presenting brain lesions at 21 & 35 dpi had still adults in the intestine and had been infected by 400 larvae. No larvae were observed in the lesions; one small granuloma was seen at 28 dpi in a mouse infected by 1200 larvae. The mechanism of brain complications during trichinellosis seems to be from hemorrhagic origin and is certainly linked to the vasculitis induced by migrating larvae and/or the toxic products of eosinophiles. This model could be used for further experiments particularly to determine the immune factors involved in brain trichinellosis.

Jean DUPOUY-CAMET, PhD
PARIS DESCARTES UNIVERSITY
TRICHINELLA NATIONAL REFERENCE CENTER
HOPITAL COCHIN APHP
27 Fbrg St Jacques
75014, Paris
France
Phone: 33 1 58 41 22 49
e-mail: jean.dupouy-camet@cch.aphp.fr
Web: http://monsite.wanadoo.fr/cnrdestrichinella/

42. Passage cultivation and utrastructure of *Trichinella spiralis* muscle larval cells cultured in vitro

Li W.Q., Z.Q. Wang, R. Wang, X.J. Hou, G.H. Zhao, J. Cui

Muscle larvae of *Trichinella spiralis* were isolated by digestion method, the cells were separated through homogenization, the primary cells were cultured in RPMI1640 medium containing 10% fetal bovine serum, and passage cultivation was carried out by trypsin (containing 0.02% EDTA). The cellular ultrastructure was observed by using transmission electron microscope, the cultured cells were identified by multiplex PCR. The results showed that the primary cells began to adhere to the substratum of culture flask 24~72 hours after inoculation. The monolayer cells were formed 12 days after inoculation, the distinct fusion phenomena among cells was not observed. One passage could be completed within 10~12d. The cells in multiplication stage were occasionally found. The results of transmission electron microscopy showed that the cell nucleus of *T. spiralis* muscle larvae are elliptic. There were clear karyotheca, nucleolus and abundant chromatin in nucleus, and plentiful mitochondria in cytoplasm. There were mainly three types of cells: elliptic, polygonal and cells with flagellum, most of them were elliptic. The band (173bp) from the cultured cells was amplified by multiplex PCR and the band was the same as *T. spiralis* muscle larvae. The above results show that the passage cultivation of *T. spiralis* muscle larval cells could be maintained in RPMI1640 medium containing 10% fetal bovine sera.

Z.Q. Wang, Professor
Medical College, Zhengzhou University
Department of Parasitology
Daxue Road 40
450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: wangzq@zzu.edu.cn

43. Humoral immune response of mice infected with low doses of muscle *Trichinella spiralis* larvae

Reiterová, Katarína, Daniela Antolová, Zuzana Hurníková

Parasitological Institute SAS
Department of parasitozoonoses
Košice, Slovak Republic

In epidemiological surveys, serological techniques are frequently used to detect parasitostatus of important reservoir hosts of zoonotic diseases. Small mammals present the most important epidemiological chain in the spread of trichinellosis. In experimental studies, high infective doses, ranging between 200 and 500 muscle larvae (L1) per mouse, are used to provoke strong immune response of laboratory animals. However, wildlife animals could be infected even by a low number of *Trichinella* spp. larvae. Therefore, the aim of this work was to reveal the size of infective doses that can evoke adequate immune response with detectable level of specific antibodies in mice. Totally 60 inbred Balb/c mice with 50 L1 and 60 outbred ICR mice with 5 L1 *T. spiralis* were infected. The total larva burdens (TLB) in the intestinal and muscle phase and reproduction capacity index (RCI) were recorded. The dynamics of specific antibodies by ELISA with three different conjugates were determined. In the first days post infection more adults were found in the intestines of Balb/c mice. Their numbers then decreased to a level similar to that of ICR mice intestines. In both mice strains, the first muscle larvae were observed on 20 dpi. Reproduction capacity index (RCI) was significantly higher in ICR mice. Seroconversion of IgM antibodies was detected on 30 dpi. The IgG antibodies appeared on 40 dpi in inbred mice, and on 50 dpi in ICR mice. Using polyvalent conjugate, the earliest seroconversion was recorded on 30 dpi. Our results confirm the suitability of ELISA in large epidemiological surveys in naturally infected small mammals. This fact is conducive in study of epidemiological relations in sylvatic circulation of trichinellosis, in order to reveal reliable ways of its transmission.

Work supported by the Slovak Grant Agency for the Science and Development Contract No. APVV-51-027 605 and partially supported by the Slovak Grant Agency VEGA, Grant No. 2/7186/27.

Katarína Reiterová, Dipl.Eng., PhD.
Parasitological Institute SAS
Department of parasitozoonoses
Hlinkova 3, 040 01 Košice
Slovak Republic
Phone: +421 55 63 344 55
e-mail: reiter@saske.sk

44. Non-canonical spliced leader trans-splicing in *Trichinella spiralis*

Connolly, Bernadette, J. Pettitt, B. Muellller, I. Stansfield

Spliced leader trans-splicing, which involves the addition of a short sequence (SL) from a non-coding RNA (SL RNA) to the 5' ends of multiple pre-mRNAs during the maturation process, was first discovered in the kinetoplastid protozoan *Trypanosoma*. Since then it has been identified in several eukaryotic phyla including the nematodes, platyhelminthes, rotifers, cnidarians and tunicates. Within an individual phylum the occurrence of SL trans-splicing is sporadic and one of the major unresolved questions concerns the evolutionary origin of SL trans-splicing. Two competing hypotheses have been offered to explain its erratic phylogenetic distribution: first that SL trans-splicing was present in an ancestral eukaryote and has subsequently been lost from most lineages; second that it evolved independently in multiple lineages. Within the phylum nematoda SL trans-splicing is widespread in the order Rhabditida and has been most extensively studied in *Caenorhabditis elegans* and *Ascaris suum*. Furthermore, the sequence of the predominant SL (SL1) is conserved throughout the order. *Trichinella spiralis* belongs to the Dorylaimia and is separated from *C. elegans* by the oldest known divergence within the phylum. We present recent data that indicates that SL trans-splicing occurs in *T. spiralis* but contrasts dramatically with that in the other nematodes studied to date. These data suggest that SL-trans-splicing is an ancient feature of nematodes.

Bernadette Connolly, PhD.
University of Aberdeen, School of Medical Sciences
Foresterhill
AB25 2ZD Scotland UK
Phone: 44 1224 555825
e-mail: b.connolly@abdn.ac.uk

45. Screening of DNA binding protein genes from newborn larvae of *Trichinella spiralis*

Hongkuan, Deng[1], Xiuping Wu[1], Xuelin Wang[1], Lianrui Li[1], Ying Zhao[1], Lei Sun[1], Shenye Yu[1], Mafeng Liu[1], Xiangye Liu[1], Yanling Yu[1], P. Boireau[2], Mingyuan Liu[1]

[1]Key Laboratory of Zoonoses, Ministry of Education, Jilin University, Institute of Zoonoses,Changchun, P. R. China
[2]JRU BIPAR INRA AFSSA ENVA UPVM, 94703 MAISONS-ALFORT cedex, France

Adult and newborn larvae of *Trichinella spiralis* (*T. spiralis*) were collected. Total RNA was extracted by Trizol reagents. Messenger RNA was isolated from total RNA by Oligotex Centrifugalization. Then the T7 phage display cDNA library of adult and newborn larvae of *T. spiralis* was constructed. The primary library capacity of library was 3×10^5 pfu, recombinant ratio was 98%, the ranges of the inserts were 250-2000bp, and the titer of amplification library was 3×1012 pfu/mL. The phage display cDNA library was screened by using mouse muscle chromosome DNA which was fixed on the nitrocellulose membrane. Following four rounds of screening, the selected phages were sequenced and analyzed. Total 13 positive clones representing 7 individual genes, in which, two clones commonly encode a homologue of *T. spiralis* hypothetical ORF 11.30. Five clones commonly encode a homologue of *Trichinella pseudospiralis* nudix hydrolyase. Two clones commonly encode a homologue of Caenorhabditis briggsae hypothetical protein CBG23797. One clone commonly encode a homologue of *T. spiralis* TGF-beta-like ligand precursor. Three clones commonly encode a homologue of Malaria parasite three different hypothetical protein ([XP_001348158], [XP_724987], [XP_726451]). The work laid the foundation for the study of the T. spiralis cyst formation mechanism and signal transduction between parasites and hosts.

Work founded by China 863 program 2006AA02Z451

Hongkuan Deng
Key Laboratory of Zoonoses, Ministry of Education, Jilin University, Institute of Zoonoses
Xian Road 5333
130062 Changchun P. R. China
Phone: +8643187836713
e-mail: trichinella@163.com
Web: http://www.jluhp.edu.cn/zuzhijigou/yanjiusuo/index.htm

46. Multiple isoforms of the secreted serine protease Tsp-SP1 are encoded by a multi-copy gene family

Krystyna Cwiklinski, Bernadette Connolly

Serine proteases are among the prominent glycosylated proteins found in the excreted secreted (ES) fraction of the Trichinella spiralis muscle larva. In 2-dimensional electrophoretic (2-DE) analysis of the ES approximately 12 peptide spots have been identified as the serine protease Tsp-SP1. These can be broadly divided into three distinct groups based on relative molecular mass and pI. The gene encoding Tsp-SP1 has been previously cloned and expression shown to be restricted to the muscle larva. Following the recent availability of the *T. spiralis* genome sequence draft assembly we have analysed of the genomic structure and organisation of the gene. BLAST analysis indicates that TspSP1 is encoded by a multi-copy gene, with three tandemly repeated copies, all of which are expressed in the muscle larva. Although the genes differ in length, the intron/exon organisation is conserved over the first five exons and within this region the copies share 82-88% nucleotide sequence identity. In each, the first five exons encode an N-terminal signal peptide and the pro-protein including the catalytic triad. The genes differ in organisation and sequence at the 3'end and thus encode proteins with alternative C-terminal domains. Recent data suggests that the three protein groups identified by 2-DE represent distinct isoforms encoded by the different gene copies. Data on the analysis of the three gene copies and on comparative analysis of the *T. pseudospiralis* homologue will be presented.

Krystyna Cwiklinski
University of Aberdeen, School of Medical Sciences
Foresterhill, Aberdeen
AB25 2ZD Scotland, UK
Phone: 44 1224 555921
e-mail: k.cwiklinski@abdn.ac.uk

47. High antigenic genes screening from cDNA libraries of *Trichinella spiralis* at different stages

Xiuping Wu[1], Baoquan Fu[2], Xuelin Wang[1], Yalan Zhang[1], Shenye Yu[1], Hongkuan Deng[1], Lei Sun[1], Xiangye Liu[1], Radu Blaga[3], Boireau P.[4], Mingyuan Liu[1]

[1]Key Laboratory of Zoonoses, Ministry of Education, Jilin University, Institute of Zoonoses Changchun, P. R. China
[2] Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, P.R. China
[3] University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania
[4] JRU BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort cedex, France

cDNA libraries of *Trichinella spiralis* (*T. spiralis*) adult (Ad, 3 days old adult) and newborn larvae (NBL) were screened using 26 dpi pig anti-T. spiralis serum. 33 positive clones representing 20 individual genes were obtained from Ad cDNA library. 4 genes have been described before, while 16 new genes were found. Interestingly, the encoded amino acid sequences of 14 clones are highly homologous with comparable score (ClustalW) from 83 to 99, and encoding 8 high similar individual homologues of serine protease-like proteins. Southern blotting and genomic structure analysis indicated these genes belong to a serine protease gene family with possible polymorphism. In the screening of NBL cDNA library, 146 positive clones representing 41 individual genes were obtained, two genes appeared high copies with 70 and 23 positive clones corresponding to Ts NBLSS2 and Hypothetical ORF 9.10 of *T. spiralis* respectively. Muscle larvae (ML) cDNA library of *T. spiralis* was screened using 60 dpi pig anti-*T. spiralis* serum, 14 positive clones representing 4 individual genes were obtained. Sequence analysis show that 10 of them commonly encoded one same gene serine protease inhibitor. All of the results lay a part of foundation for the recombinant antigens potentially used in diagnosis and vaccine of *Trichinella spiralis*.

Work founded by China 863 program 2006AA02Z451

Ying Zhao, PhD
Key Laboratory of Zoonoses, Ministry of Education, Jilin University, Institute of Zoonoses
Xian Road 5333
130062 Changchun P. R. China
Phone: +8643187836713
e-mail: wxlwqx@sohu.com
Web: http://www.jluhp.edu.cn/zuzhijigou/yanjiusuo/index.htm

48. Cloning and characterization of a potential early diagnosis gene from newborn larvae of *Trichinella spiralis*

Mingyuan Liu[1], Fu Baoquan[2], Danielle Le Rhun[3], F. Le Guerhier [3], Wu Xiuping[1], R. Hernandez Bello[3], Isabelle Vallée[3], Céline Bahuon[3], P. Boireau[3]

[1]Key Laboratory of Zoonoses, Ministry of Education Jilin University, Institute of Zoonoses
[2]Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, P.R. China
[3]JRU BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort cedex, France

A newborn larvae (NBL) stage specific serine protease gene (Termed TsNBLSS2, Patent No: PCT/FR2007/000221) was cloned from *Trichinella spiralis* (*T. spiralis*). The predicted amino acid sequence from this gene consisted of a putative signal peptide, a trypsin-like serine protease catalytic domain and a C-terminal repeated domain. Southern blotting and genomic structure analysis suggested this gene present as a single copy in the genome of *T. spiralis* with 5 introns, which showed identical intron pattern to those of trypsin or kallikrein subclass of S1A peptidase. The transcript of this gene analyzed by RT-PCR is promoted from one day old NBL to nine days old NBL. In Western blot analysis, the recombinant proteins of full encoding region and the C-terminal domain were strongly recognized by 26 dpi pig serum infected by *T. spiralis* but weak with 60 dpi serum while the recombinant protein of catalytic domain were not recognized by two kinds of serum, the corresponding band were detected in crude antigen and ES antigen of 5 days adult and newborn larvae by rabbit antiserum against recombinant protein of catalytic domain. ELISA showed that the recombinant proteins of C-terminal domain could be recognized by pig anti-*Trichinella* serum as early as 15 dpi, 20dpi and 20dpi with infection dose of muscle larvae 20000, 1000 and 200, respectively. A good candidate for early diagnosis was found.

Work founded by the EU project TRICHINET/ TRICHIMED workpackage (MedVetNet) and China 863 program 2006AA02Z451

Mingyuan Liu, Professor
Key Laboratory of Zoonoses, Ministry of Education
Jilin University, Institute of Zoonoses
Xian Road 5333
130062 Changchun P. R. China
Phone: +8643187836713
e-mail: liumy36@163.com
Web: http://www.jluhp.edu.cn/zuzhijigou/yanjiusuo/index.htm

49. Caveolin 1 and cholesterol play a major role in the develompment of *Trichinella spiralis* oocytes

Ortega-Pierres, Guadalupe[1], R. Hernández-Bello[1], Rosa M. Bermúdez-Cruz[1], R. Fonseca-Liñán[1], Patricia García-Reyna[2], P. Boireau[3]

[1]Department of Genetics and Molecular Biology, Centro de Investigación y Estudios Avanzados del IPN, Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Cuautitlan
[3]UMR BIPAR INRA-AFSSA-ENVA-UPVM, Maisons Alfort, France

Caveolins are integral membrane proteins implicated in cholesterol homeostasis and transport, endocytosis mechanisms and regulation of signal transduction in differentiated cells. We have identified a caveolin-1 gene from the nematode parasite *Trichinella spiralis* (CavTs). For this, a stage-specific cDNA library of 3-days old adult worms was screened using a stage-specific cDNA labeled-probe. A selected clone contained a cDNA insert of 1,427-bp and a full length ORF of 687-bp, which encodes for a 229 polypeptide with a MW of 26 kDa. Confocal laser microscopy analysis using antibodies against CavTs and cross-sections of *T. spiralis* adult parasites showed that CavTs is gradually accumulated in the ova and later on in the oocyte membrane, reaches a maximum expression at day 3 pi and decreases during newborn larva development. RT-PCR assays with specific CavTs primers using 1 to 4 days old parasites showed a similar gene expression profile as the one observed for CavTs suggesting a developmental regulation for CavTs gene. Free cholesterol in female worms as detected by filipin staining was mainly distributed in germ line and in the oocyte membranes as was observed for CavTs suggesting a temporal membrane association of cholesterol with CavTs for proper functions. When cyclosporin A (CsA) was given to mice during infection with T. spiralis and worms were collected at different times post infection it was observed that development of oocytes was practically abolished in female worms. Also indirect immunofluorescence staining in sections of adult worms using anti- CavTs antibodies showed that CavTs had a lower expression and an irregular distribution in the oocyte membranes in female worms collected from CsA treated rats as compared with parasites obtained from untreated control rats. All together these results suggest that CavTs plays a role in oocyte maturation during development of ML to adult parasites demonstrating a specif gender expression of this gene.

Supported in part by ECOS-ANUIES Grant M06-S03 and CONACyT Grant G38523.

Guadalupe Ortega-Pierres, PhD
Centro de Investigacion y de Estudios Avanzados del IPN
Department of Genetic and Molecular Biology
Av. Instituto Politecnico Nacional No. 2508
07360 Mexico, D.F. Mexico
Phone: 55 5061 3331
e-mail: gortega@cinvestav.mx

50. The development of the *Trichinella* nurse cell and the role of stage-specific proteins

Boireau, P.[1], L. Mingyuan[2]

[1]AFSSA, INRA, ENVA, UPVM, UMR 956 BIPAR, AFSSA LERPAZ, Maisons Alfort, France
[2]Key Laboratory of Zoonoses, Ministry of Education, Institute of Zoonoses, Jilin University, 5333 Xian Road, 130062 Changchun, P. R. China

Trichinella spiralis is a unique intracellular parasitic nematode that is distributed worldwide and can infect almost all mammals, including humans. The life cycle of *T. spiralis* is completed within a single host species and infection starts with the consumption of infective muscle larvae (ML) and digestion of the protective capsule within the host stomach. Larvae undergo four fast molts in intestinal epithelial cells and eventually develop into sexually mature adults (Ad) approximately 2-3 days post infection (pi). Freshly released newborn larvae (NBL) are carried to host tissues by blood flow and invade new host cells. The NBL penetrate striated muscle cells and undergo developmental changes. Larvae that are older than 14 days can be infective to subsequent potential hosts and may remain viable for the entire life span of the host.

To date, little is known about the molecular mechanisms that are involved in parasite development and survival within the cytoplasm of host cell. Identification of stage-specific genes will be important for elucidation of these mechanisms. ML, Ad and NBL are three major stages in the life cycle of *T. spiralis* that exhibit distinct antigenicity, indicating differential regulation of many parasite proteins. Previously, very few developmentally regulated antigens have been characterized, except the ML stage-specific TSL-1 antigens identified by monoclonal antibodies. Several genes expressed during the ML stage were identified recently and one of these genes has been speculated to be involved in nurse cell formation. Proteins synthesized in *T. spiralis* and secreted in the host cell are suspected to be involved in the process of the nurse cell in the previous studies, however, up to now, only one of them, the 43 kDa polypeptide (P43), has a helix-loop-helix (HLH) motif that have been suggestive of a function that might be relevant to the Nurse cell formation, this cDNA has been identified to encode a homologue of DNase II now. Further more, the DNase activity has also been proved to be present in the ES of Trichinella spiralis but not in it from Trichinella pseudospiralis (a non-encapsulated Trichinella species). Recently Mitreva et al, 2004 generated a *T. spiralis* expression sequence tags (ESTs) database containing 3262 unique genes from cDNA libraries of immature L1 larvae (also known as NBL), mature ML and adults (3-day-old adults, Ad3). However, the majority of the identified genes have been clustered in only one developmental stage.

Pure adult worm populations are difficult to obtain as at day 2 pi adults are still mixed with

different stages of larvae and at day 5 and 6 pi many NBL are formed in female adult womb. It has been tested that adults at day 3 pi (Ad3) represent a stage without any NBL (Liu et al., 2001), although they may contain some fecundated eggs in early stages of development (Wu et al., 2005). On the contrary, female adults contain all developmental stages (from fecundated eggs to NBL) of embryo in the womb beginning at day 4 pi. Moreover, due to technical difficulties in obtaining sufficient and pure NBL, until now only one gene has been published as NBL stage-specific (Zarlenga et al., 2002).

In an attempt to identify stage-specific genes of *T. spiralis*, subtracted cDNA libraries of NBL, Ad3 and Ad5 were constructed respectively, using a suppression subtractive hybridization (SSH) technique. A number of stage-specific cDNAs derived from NBL, Ad3 and Ad5 were identified and analyzed. Six genes were identified as NBL stage-specific, including one member of the *T. spiralis* gene family encoding glutamic acid rich proteins, two genes encoding novel serine proteases, two closely related genes encoding proteins that are members of a deoxyribonuclease II (DNase II)-like family and one nucleotidic sequence with no similarity to known genes. The twin genes encoding DNaseII (DnaseII1Ts/NBL and DnaseII2Ts/NBL) have a high percentage of identity in their amino acid (aa) sequence (89.6%), and their predicted aa sequences exhibited a N-terminal signal peptide, a potential helix-loop-helix motif and the conserved domains of DNase II. The DNase activity have been obtained with the purified recombinant proteins DnaseII1Ts/NBL. Considering that NBL directly promote the nurse cell forming of infected muscle cell the importance of DNase II in the nurse cell formation of *T. spiralis* will be discussed. Four stage-specific clones encoding homologues of retinoid X receptor, caveolin, C2H2 type zinc finger protein and a putative protein with no homology to known sequences were obtained from 3-day-old adult worms. The caveolin-1 gene (CavTs) was characterized and identified as an adult-specific antigen. CavTs is gradually accumulated only on the ova surface reaching a maximum at 3 days pi, and decreasing during newborn larva (NBL) development. Reverse transcriptase polymerase chain reaction (RT-PCR) assays of parasites from 1 to 4 days pi showed a similar gene expression profile that observed for CavTs, which suggests a specific developmental regulation. Another target (AdTs1) was analysed in this 3-day-old adult subtractive cDNA library. The selected gene encodes a protein with two putative zinc finger domains. Interestingly, some strong similarities were found between the AdTs1 protein, nuclear hormone receptors of mammals or other species and a C. elegans gene, nhr-2 that was characterised as a member of the nuclear hormone receptor family strongly expressed during the embryogenesis. The NHR-2 protein was located earlier in embryonic nuclei until the 16-20 cell stage in C. elegans. The high abundance of AdTs1 mRNA during the 3 day-old Ad stage may suggest that this can be homologous to nhr-2 of C. elegans.

One gene specifically up-regulated in the 5-day-old adult worms encoding a putative cuticle collagen was also identified.

During the analysis of the subtractive cDNA library additional clones were selected that could be relevant in the biology of *Trichinella*. But all these clones are expressed during all the Trichinella developmental stages some of them having more intense transcription during a period

of *Trichinella* life. So, nucleotidic sequence of the GST24 gene of various *Trichinella* species was obtained. The open reading frame coding for GST24Ts was expressed in a prokaryotic system and purified by glutathione-affinity columns. GST24Ts enzymatic activity was proved to be different from the activity of mammalian pi class GSTs, and other pi class related nematode GSTs. With the universal substrate 1-chloro-2, 4-dinitrobenzene (CDNB), the specific activity of GST24Ts was 3.79 µmole.min-1(mg protein)-1. The tissue distribution of GST24Ts was examined by immunohistochemistry in muscular larvae (ML) of *T. spiralis* and the nurse cell. GST24Ts was located exclusively in the stichocytes and the genital primordium without any export in the cytoplasm of the nurse cell.

In brief the *Trichinella* developmentally regulated genes that has been described, can be divided into two main functions: i) the development of the parasite (CavTs AdTs1) ii) the interaction with the host cell (proteases, DNaseII) to allow the generation of the nurse cell. The data generated from this study have provided new knowledge to our understanding of *T. spiralis* gene expression mechanisms during various developmental stages. These findings have provided information of *Trichinella* parasite biology and pathogenesis, which will pave the way for the development of more specific and sensitive immunodiagnostic technologies and for the prevention of trichinellosis.

Part of this work was supported by the EU contracts TRICHIPORSE QLRT-2000-01156 MedVetNet (Food-CT-2004-506122) WP11 Trichinet and WP27 Trichimed and the grant of China 863 program 2006AA02Z451

Pascal Boireau, PhD
AFSSA, INRA, ENVA, UPVM
JRU BIPAR, AFSSA LERPAZ
AFSSA LERPAZ
23 av du Général de Gaulle
94700 Maisons Alfort
FRANCE
Phone: 33 1 49 77 13 00
e-mail: p.boireau@afssa.fr

51. Evaluation of the protection induced by the 30-mer peptide from *Trichinella spiralis* 43-kDa antigen expressed on the surface of attenuated Salmonella enterica in experimental murine trichinellosis

Ortega-Pierres, Guadalupe [1], R. Fonseca-Linan[1], Aida Castillo-Alvarez[1,2], N. Villegas-Sepulveda[2], Lilian Yepez-Mulia[3]

[1]Departments of Genetics and Molecular Biology
[2]Molecular Biomedicine, Centro de Investigacion y de Estudios Avanzados del IPN
[3]UIMEIP-Pediatria, IMSS. Mexico

Infection with *Trichinella spiralis* induces strong local and systemic immune responses in infected hosts. Several studies have shown that parenteral immunization of mice with *T. spiralis* antigens induces protective responses upon challenge with the parasite. However few studies have focused on the evaluation of host mucosal immunization with *T. spiralis* antigens. Recently successful immunization of mice against *T. spiralis* by intranasal administration of a 30-mer peptide from the 43 kDa antigen and cholera toxin B was reported. The possible use of this peptide in large scale prophylaxis requires however other delivery systems. In this context, the attenuated Salmonella enterica may serve as live vector to deliver foreign antigens to the immune system by expressing them through prokaryotic expression plasmids. Recently we have expressed the *T. spiralis* 30-mer peptide on the surface of *Salmonella typhimurium* SL3261 (ARO A-) by the use of the C- terminal domain of the autotransporter MisL of *Salmonella enterica*. Analysis of bacterial extracts by SDS-PAGE and Western blot showed an over expression of a fusion protein of ~70 kDa. Localization of the peptide on the bacterial surface was confirmed by immunofluorescence assays. Intranasal immunization of mice with this live vector expressing the 30-mer peptide induced a significant protection against *T. spiralis* infection. The protective effect was improved by an intraperitoneal boost with the recombinant peptide. These results suggest that the 30-mer peptide and the vector used may be potential candidates to elicit mucosal immunity to *T. spiralis*. (Partially supported by CONACyT grant No-G38523-M)

Guadalupe Ortega-Pierres, PhD
Centro de Investigaciony de Estudios Avanzados del IPN
Department of Genetic and Molecular Biology
Av. Instituto Politecnico Nacional No. 2508
07360 Mexico, D.F. Mexico
Phone: 55 5061 3331
e-mail: gortega@cinvestav.mx

52. Prokaryotic Expression of the Antigen Gene Ts 21 of *Trichinella spiralis* and Identification of its Recombinant Protein

ZQ Wang, Li Lu, J Cui, L Wang, R Wang

To express the antigen gene Ts21 of Trichinella spiralis, purify the recombinant protein and identify its antigenicity, the gene was sub-cloned into the prokaryotic expression vector pMAL-c2X and the recombinant pMAL-c2X-Ts21 was transformed into an *E. coli* TB1 strain and induced by IPTG. The expression products were purified by MBP-binding affinity chromatography. Mice were immunized with the purified recombinant protein, the titers of the immune sera were assayed by ELISA and the distribution of Ts21 protein in muscle larvae was observed by Indirect immune fluorescent test (IFT). The results showed the expressed fusion protein was about 63.5 kDa and the expression level peaked at 4 h post-incubation. On Western blot analysis, the recombinant protein was recognized by sera from mice infected T1 and T7 as well as patients with trichinellosis, but not recognized by sera from mice infected with T2, T3 and T4. The recombinant protein did not react with sera from patients with ancylostomiasis, cysticercosis and schistosomiasis, but cross-reacted with sera from patients with paragonimiasis, clonorchiasis and echinococcosis. High titers of antibodies were produced in mice immunized with the recombinant protein. IFT showed the Ts21 protein was mainly distributed in the cuticle of muscle larvae. The work was supported by the National Natural Science Foundation of China (30471450) and Henan Innovation Project for University Prominent Research Talents (2004KYCX013).

ZQ Wang, Professor
Medical College, Zhengzhou University
Department of Parasitology
Daxue Road 40,
450052 Zhengzhou China
Phone: 86-0371-66181026
e-mail: wangzq@zzu.edu.cn

53. Molecular cloning and characterization of a novel gene from *Trichinella spiralis*

Jing Yang[1], Xinping Zhu[1], Yaping Yang[1], Yuan Gu[1], Qiang Li[1], Junfei Wei[1], Shaohua Wang[1], P. Boireau[2]

[1]School of Basic Medical Sciences, Capital Medical University, Department of Parasitology, Beijing, China
[2] UMR 956 INRA-AFSSA-ENVA-UPVM, 94703 Maisons-Alfort, France

The adult cDNA expression library of *T. spiralis* was immunoscreened with the sera from infected rabbits. A novel gene encoding a sequence homologous to paramyosin was cloned. The full length cDNA sequence of *T.spiralis* paramyosin (TsPmy) was amplified and cloned into the prokaryotic expression system. The recombinant protein with His-tag was purified by Ni-affinity chromatography. Western blot using the recombinant TsPmy, showed that the sera from patients, infected swine, rabbit and mice were strongly reactive against the protein, respectively. The antibody against the recombinant TsPmy positively bound to the crude extracts of muscle larvae and adult worm by Western blot. BALB/c mice immunized with TsPmy demonstrated 32% reduction in worm burden. The vaccine induced the production of high levels of specific IgG1 antibodies and the significant levels of IgG2a were also detected. The results of cytokine production showed that the significant elevation in IL-4 and IL-10 release was observed, but low production of IFN- and IL-2 was also detected. These studies showed that the recombinant TsPmy generated a mixed Th1/Th2 of immune responses, with the Th2 predominant, and induced partial immune protection against T.spiralis.

The project was supported by the grants from Beijing Municipal project for Developing Advanced Human Resources for Education (BAHED) and the Ph.D. Programs Foundation of Ministry of Education of China (20060025003).

Xinping Zhu
School of Basic Medical Sciences
Capital Medical University
Department of Parasitology
No. 10 Xitoutiao, Youanmen
Post Code: 100069 Beijing China
Phone: 86-10-83911436
e-mail: zhuxping@ccmu.edu.cn

54. Identification and characterization of the full-length cDNA encoding paramyosin of *Trichinella spiralis*

Xinping Zhu[1], Jing Yang[1], Yaping Yang[1], Yuan Gu[1], Qiang Li[1], Junfei Wei[1], Shaohua Wang[1], P. Boireau[2], Xinping Zhu[1]

[1]School of Basic Medical Sciences, Capital Medical University, Department of Parasitology, Beijing China
[2]UMR 956 INRA-AFSSA-ENVA-UPVM, Maisons-Alfort, France

A clone was isolated from the cDNA library of adult worm of *T. spiralis* through immunoscreening by infected rabbit sera. The clone contained a cDNA transcript of 2568bp in length with a single open reading frame, which encoded 885 amino acid (102kDa in the estimated molecular weight). A sequence analysis revealed that this clone encoded the full-length of paramyosin gene, and the clone was designated as TsPmy. The fusion proteins encoded by the TsPmy were produced in an *E. coli* expression system and purified by Ni-affinity chromatography. Western blot using the recombinant TsPmy, showed that the sera from patients, infected swine, rabbit and mice were strongly reactive against the protein, respectively. The antibody against the fusion protein positively bound to the 110kDa band (with the histidine tag) in crude extracts of muscle larvae and adult worm by Western blot. BALB/c mice vaccinated with recombinant TsPmy demonstrated 32% reductions in worm burdens. Vaccination of the mice resulted in high levels of specific anti-TsPmy IgG antibodies and generated a Th1/Th2 mixed type of immune responses. These studies showed that the recombinant TsPmy can induce immune response in mice. The project was supported by the grants from Beijing Municipal project for Developing Advanced Human Resources for Education (BAHED) and the Ph.D. Programs Foundation of Ministry of Education of China (20060025003).

Xinping Zhu
School of Basic Medical Sciences
Capital Medical University
Department of Parasitology
No.10, Xitoutiao, Youanmenwai
100069 Beijing, China
Phone: 86-10-83911436
e-mail: cnu_yangjing@yahoo.com.cn

55. Cloning, expression and identification of a novel 70-KDa heat shock protein gene (Ts-hsp70) gene from *Trichinella spiralis*

Wang Shaohua[1], Xinping Zhu[1], Yaping Yang[1], Jing Yang[1], Ran Hao[1], P. Boireau[2]

[1]School of Basic Medical Sciences, Capital Medical University, Department of Parasitology
[2]UMRINRA-AFSSA-ENVA, Maison-Alfort, Paris, France)

A novel gene from *Trichinella spiralis* (T.s) was got by screening the adult cDNA library of T.s using the sera from infected rabbits. The cDNA full length of the clone was obtained and designated as Ts-hsp70. The sequence of Ts-hsp70 was analyzed by DNAstar software and GenBank database. Ts-hsp70 has an open reading frame of 1,674 nucleotides encoding 558 amino acids residues. The deduced amino acid sequence of Ts-hsp70 showed high identity with 70KDa heat shock proteins of some other parasites. Ts-hsp70 was amplified by PCR and cloned into the prokaryotic expression vector pET-28a(+). The transformants were induced by IPTG . The expression products were analyzed by SDS-PAGE and purified by His-Bind column. Western-blotting showed that the purified recombinant Ts-hsp70 could be recognized by anti-His antibody and the sera from infected mice. Furthermore BALB/c mice were vaccinated with the recombinant Ts-hsp70 and the protective effect was studied by a challenge experiment. The mice immunized with the recombinant Ts-hsp70 showed 37% worm burden reduction. ELISA showed higher specific antibody titer induced by the recombinant Ts-hsp70 in mice. It suggested that the recombinant Ts-hsp70 induced partial immune protection against T.s in BALB/c mice. The project was supported by the grants from the National Natural Science Foundation of China (No.30571626) and the Natural Science Foundation of Beijing (No.5062005).

Xinping Zhu
School of Basic Medical Sciences
Capital Medical University
Department of Parasitology
No.10, Xitoutiao, Youanmenwai
100069 Beijing China
Phone: 86-10-83911436
e-mail: zhuxping@ccmu.edu.cn

56. Increased expression of a new class of glutathione-s-transferase, named omega, in the nurse cell during *Trichinella britovi* infection as revealed by in situ hybridisation

Bruschi, F.[1], Simona Piaggi[1], Alessandra Salvetti[2], Lorena Chiumiento[2], A. Casini[2], V. Gremigni[2]

[1]Dept. of Experimental Pathology, MBIE
[2]Departement of Human morphology and Applied biology, Universitŕ di Pisa, Italy

Glutathione-S-Transferases constitute a family of multifunctionally enzymes utilizing glutathione in reactions which biotransform a wide range of exogenous and endogenous compounds. Recently, a new class of GST named Omega (GSTO) has been identified. The GSTOs show a series of properties which characterize them as stress response proteins. They are found in a wide range of species including human, rat and mouse. Among the different aspects of the host-parasite relations at muscle level in trichinellosis, the role of anti-oxidant system have rarely been studied to date. To better elucidate these aspects, we investigated the ability of the muscle cell infected with Trichinella britovi to produce the mouse GSTO1 (previous known as DHAR and p28) and the recently identified GSTO2. Biochemical data, immunoblot analysis and immunohistochemical studies show an overexpression of GSTO in the nurse cell (NC) compared to the surrounding muscle fibres. To evaluate the expression level of GSTO1 and GSTO2 we performed in situ hybridization at different infection times. Diaphragms from mice at 20, 40 and 60 days of infection with *T. britovi* were isolated and processed for the "in situ" hybridisation. The results show that no relevant amount of GSTO1 and GSTO2 are present in the parasite but there is an increasing stain with the progression of infection as regards the GSTO1 probe exclusively in the NC, whears for the GSTO2, preliminary results show too low level of GSTO2 mRNA to be revealed.

Fabrizio Bruschi, Professor
Universita' di Pisa
Department of Experimental Pathology, M.B.I.E.
Via Roma 55
56126 Pisa Italy
Phone: +39(050)2218547
e-mail: fbruschi@med.unipi.it

57. Detection of O-glycosylated proteins from different *Trichinella* species muscle larvae total extract. A lectin affino-blot study

Rositsa Milcheva[1], L. Mihov[1], E. Pozio[2], P. Babál[3]

[1]Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Department of Infectious, Parasitic and Immunomediated Diseases, Instituto Superiore di Sanita, Rome, Italy
[3]Department of Pathology, Faculty of Medicine Comenius University, Bratislava, Slovakia

Rositsa Milcheva
Department of Biochemistry,
Institute of Experimental Pathology and Parasitology - Bulgarian Academy of Sciences,
Acad. G. Bonchev Str., Block 25,
Sofia 1113,
Bulgaria
tel. +359 2 979 23 66;
e-mail: rosicamilcheva@abv.bg

The aim of the work was to analyze oligosaccharide composition with the focus on O-linked glycoproteins presence in the total extract obtained from different *Trichinella* species muscle larvae by means of lectin affino-blot with lectins selected to their sugar specificity. The absence of reactivity with two lectins-TML and MAL indicated that the species from the *Trichinella* genus do not synthesize sialic acid. Reactivity with HPA, VVL-B4, PNA and UEA-I suggested expression of O-linked glycans identical to carcinoma-associated Tn-antigen (GalNAc-α-Ser/Thr) and T-antigen (Gal-β1,3-GalNAc-α-Ser/Thr) and also structures analogous to A-blood group antigens (GalNAc-α1,3-Gal-β1,3(4)-(Fuc-α1,2-)-R). The obtained results may contribute to a better understanding of the glycobiology of this parasitic nematode in relation to occupation of its intracellular niche.

58.

Sylvatic trichinella infection in Finland

Saari, S.[1], Niina Airas[1], Anna-Maija Virtala[1], Taina Mikkonen[1], Pellikka Jari[1], A. Oksanen[2], Marja Isomursu[2], A. Sukura[3]

[1]Department of Forest Ecology, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland
[2]Oulu Research Unit, Finnish Food Safety Authority Evira (FINPAR), Oulu, Finland
[3]Department of Basic Veterinary Sciences (FINPAR), Faculty of Veterinary Medicine, University of Helsinki

The prevalence of sylvatic trichinella infection in Finland varies by region. It is hypothesized that the raccoon dog acts as an important reservoir for trichinella in Finland. However, due to the adverse winter, raccoon dogs do not survive in northern Finland where trichinella prevalence is comparatively reduced relative to southern Finland.

The material consisted of 2401 animals (1110 foxes, 662 raccoon dogs, 402 lynx, 125 brown bears, and 102 wolves) collected by hunters between 1999 and 2005 from different geographical locations in Finland. Muscle samples from each animal were artificially digested to determine the density of trichinella infection. *Trichinella* spp. was isolated from 625 animals. The prevalence in red foxes was 19%, 45.3% in lynx, 5.6% in bears, 38.2% in wolves, and 28% in raccoon dogs.

From the total amount of positive samples by digestion, the specific *Trichinella* species was identified in 324 samples by multiplex-PCR. *T. nativa* was observed in 80% of PCR positive samples, *T. spiralis* in 15.3%, *T. britovi* in 9%, and *T. pseudospiralis* in 2.3%. Seven percent of the animals were infected with two different trichinella species; the most common combination being *T. native* and *T. spiralis* (8% of mixed infections).

The study confirms that sylvatic trichinella infection is common in Finland; also all four trichinella species previously described in Finland were detected by multiplex-PCR. Additionally, the raccoon dog seems to play a fundamental role in the epidemiology of trichinellosis in Finland.

Antti Sukura, Professor
Faculty of Veterinary Medicine, University of Helsinki
Department of Basic Veterinary Sciences (FINPAR)
P.O.Box 66
FI-00014 Finland
Phone: +358919157150
e-mail: antti.sukura@helsinki.fi

59.

Long-term survey on trichinellosis in wildlife of Slovakia

Hurníková, Zuzana, Emilia Dvoroznakova

Parasitological Institute SAS, Kosice, Slovak Republic

In Slovakia monitoring on the prevalence of *Trichinella* spp. in wildlife has been performed since 2000 in main reservoirs – the red fox (*Vulpes vulpes*) and wild boar (*Sus scrofa*) using artificial digestion method recommended by ICT. Multiplex PCR approach was used for species determination. The results of investigation performed in 4669 red foxes showed that vulpine trichinellosis is widespread across Slovakia and the prevalence increased from 4.9 % in 2000 up to 13.0 at present. In recent also moderately more frequent occurrence in wild boars was recorded (0.11 %) with findings of infected animals also beyond endemic locality. The results indicate that in spread of parasite is involved also wild boar, although in maintenance of sylvatic cycle plays this host only secondary role. *T. britovi* is the dominant species circulating in Slovakia, both in foxes and wild boars, *T. spiralis* occurs only sporadically. In one wild boar from Eastern Slovakia mixed infection of *T. britovi* and *T. pseudospiralis* was recorded. This finding is considerable in relation to evidence of *T. pseudospiralis* on pig farm in the same district two years ago. The presented study provides a complex picture on *Trichinella* – occurrence in all regions of Slovakia and may be instrumental as a base for evaluating the risk of infection in domestic cycle and humans.

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-51-010704.

Zuzana Hurníková, DVM
Parasitological Institute SAS
Hlinkova 3, 040 01 Košice
Slovak Republic
Phone: ++421 55 6334455
e-mail: hurnikz@saske.sk

60. Studies on feeding habits and parasitological status of red fox, golden jackal, wild cat, stone marten, wild boar and badger from Sredna gora, Bulgaria

Kirkova Zvezdelina[1], E. Raychev[2], Dimitrina Georgieva[3]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria
[2]Department of Game animals, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria
[3]Department of Parasitology, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

In the period 2001-2006 the feeding habits of 167 foxes, 78 jackals, 40 wild cats and 23 stone martens from the area of Sredna Gora, Bulgaria were studied. 133 of the foxes, 56 of the jackals, 22 of the wild cats and 21 of the martens were subjected to helminthological study. 147 wild boars and 26 badgers from the same area were subjected to trichinelloscopy.

Rodents were the main food of the wild cats (82, 7%), martens (52%) and foxes (50%). The main food of the jackals was carrion from domestic and wild animals (79,5%). 95,5% of the foxes, 100% of the jackals, 95,5% of the wild cats and 89% of the stone martens were infected with one or more helminth species. The prevalence of the most important helminths: *Trichinella* spp., *Taenia* spp. and *Ancylostoma* spp. was high in all carnivores examined. 45, 5% of the wild cats, 40% of the jackals, 29, 5% of the foxes and 28, 6% of the martens were found to be infected with *Trichinella* spp. Isolates obtained from 15 foxes, 10 jackals, 6 stone martens and 5 wild cats naturally infected with *Trichinella* spp. were subjected to speciation by both multiplex PCR and cross-breeding experiments. In the infected with *Trichinella* spp. animals only T. britovi was demonstrated. The wild boars and badgers were not infected with Trichinella spp.

The correlation between the feeding habits and parasitological status is discussed.

Zvezdelina Kirkova
Department of Parasitology
Faculty of Veterinary Medicine, Trakia University,
Stara Zagora, Bulgaria
e-mail: z.t.kirkova@abv.bg

61.

Trichinellosis in wild and domestic animals in Poland

Cabaj, W., Božena Moskwa, Katarzyna Gozdzik, Justyna Bien

Witold Stefanski Institute of Parasitology of the PAS, 00-818 Warsaw, Twarda 51/55, Poland

Till now, 4 species genus *Trichinella* have been identified in Europe. The aim of the investigation has been to study distribution of *Trichinella* species in animals from Poland. Muscles were collected from red foxes (*Vulpes vulpes*), wolves (*Canis lupus*), wild boars (*Sus scrofa*), raccoon dogs (*Nycterutes procyonoides*) killed by hunters in Poland from 1995 to 2005 and from slaughtered domestic pigs in which larvae of *Trichinella* (ML) were detected. ML were collected after standard artificial digestion and preserved in 75 % ethyl alcohol before identification. The molecular identification of *Trichinella* larvae at the species level has been carried out at the ITRC in Rome, Italy. Out of 77 *Trichinella* isolates from red foxes; 52 resulted *T. britovi*, 6 *T. spiralis*, 4 mixed infection with both species and 15 were not identified. On 10 examined wolves from Bieszczady region 6 animals resulted positive for *T. britovi* larvae. Out of 106 isolates from wild boars; 19 resulted *T. britovi*, 78 *T. spiralis*, 3 mixed infection and 6 were not identified. On 6 raccoon dogs two were infected *T. spiralis*. Out of 21 isolates from domestic pigs; 20 resulted *T. spiralis* and 1 *T. britovi*. The study showed, that up to now, two *Trichinella* species are present in Poland; *T. britovi* as the important etiological agent of sylvatic trichinellosis especially in carnivores in almost the whole territory of Poland. *T. britovi* is also present in wild boars but in minority and very occasionally in domestic pigs.

Cabaj Wladyslaw, PhD, Professor
Witold Stefanski Instiute of Parasitology
Polish Academy of Sciences
51/55 Twarda Street
00-818 Warsaw,
Poland
Phone: + 48 22 620 62 26
e-mail: cabajw@twarda.pan.pl
Web: iparpas@twarda.pan.pl

62. *Trichinella* surveillance system in wild and domestic animals in France. Outcome of the last 10 years

Boireau, P.[1], Isabelle Vallée[1], Sophie Rossi[2], J. Hars[2], J.D. Camet[3]

[1]NRL Parasites, JRU BIPAR, AFSSA LERPAZ, Maisons Alfort, France
[2]ONCFS. Unité sanitaire de la faune. 5 Allée de Bethléem 38610 F-Gičres, France
[3]NRC for Trichinella, Hôpital Cochin, René Descartes University, Paris, France

In 1998 two serious human trichinellosis outbreaks occurred in France after the consumption of raw horse meat. An improvement of the diagnostic test in routine laboratories was applied immediately under the coordination of the National Reference Laboratory (NRL). 1) Increase the size of the horse meat sample to be digested (10g) 2) a control by two independent routine laboratories for horses. A quality plan was developed in three steps: 1) the technicians and the responsible of each routine laboratory should follow a specific training by the NRL 2) Ring trial tests should be followed by all the routine laboratories (period 2003-2007) 3) accreditation of the test (2008). The efficiency of such measures are analyzed. Since 1998 two horse carcasses were destroyed before human consumption. The emergence of *Trichinella* in pig meat was detected in Corsica and several wild boar carcasses were also blocked after the identification of *Trichinella* or other parasites in meat. A serological survey was conducted in wild boar population during the last 4 years (more than 5000 samples analyzed). Some areas exhibited highest apparent prevalence (more than 8%) with the report of human outbreaks (CNR *Trichinella*) due to the consumption of uncontrolled wild boar meat. The implementation of the quality plan allowed to reduce the pressure on horse meat control but maintaining the same security for consumer.

Part of this work was supported by ONCFS/DGAL and Trichimed (Med Vet Net) contracts

Pascal Boireau, PhD
AFSSA, INRA, ENVA, UPVM, AFSSA LERPAZ
Department of NRL Parasites, JRU BIPAR
23 av du Général de Gaulle,
94700 Maisons Alfort FRANCE
Phone: 33 1 49 77 13 00
e-mail: p.boireau@afssa.fr

63. Trichinellosis in domestic swine and wildlife in Finland after 1993

Leena Oivanen[1], Antti Oksanen[2]

[1]Finnish Food Safety Authority Evira, Mustialankatu 3, FIN-00790 Helsinki, Finland
leena.oivanen@evira.fi
[2]Finnish Food Safety Authority Evira, P.O.Box 517, FIN-90101 Oulu, Finland
antti.oksanen@evira.fi

At ICT8 in 1993, we reported an increase of Trichinella infection prevalence in slaughter swine in Finland. The situation started in the early 1980s and continued also after that report, so that the highest number of infected swine was registered in 1996.

During the last ten years (1997-2006) the prevalence of *Trichinella* infection in Finnish pigs has been markedly decreasing, with the latest detected cases being from 2004 . Slaughtered pigs are always tested for *Trichinella*, regardless of if intended for export or domestic consumption. However, the prevalence of trichinellosis in wildlife has remained at a high level. All the revealed *Trichinella* infections of pigs are analysed for species by multiplex PCR. For the time being, all larvae from pig infections have been identified as Trichinella spiralis. At the same time period, the number of pig farms has decreased, too, while the yearly number of pigs slaughtered has been stable or perhaps even slightly increased.

No change in the high *Trichinella* prevalence in Finnish wildlife has been seen, with both foxes, raccoon dogs, wolves and lynx in the southern part of the country commonly infected. The most common species in wildlife is *Trichinella nativa*, the species with very low infectivity to swine, but also *T. spiralis*, *Trichinella britovi* and *Trichinella pseudospiralis* do occur.

The most obvious explanation to the decrease in *Trichinella* prevalence and incidence is the change in Finnish swine industry since Finland joined the EU in 1995. Productivity has become increasingly crucial to the producer, which has forced the smaller, often less hygienic family farms to termination of production. New piggeries are typically large scale enterprises with corporative ownership. The new facilities obviously protect pigs better against *Trichinella* infection present in wildlife in the surroundings.

If the trend does not change in the future, maybe also Finland will some day be able to apply for status of region with negligible *Trichinella* risk.

64. *Trichinella* sp. infection in horses of Romania: serological survey and parasitological examination

Blaga, R[2,7]., Carmen M Cretu[1], C. Gherman[2], Alina Draghici[3], E. Pozio[4], K. Noeckler[5], C.M.O. Kapel[6], I. Dida[3], V. Cozma[2], P. Boireau[7]

[1]"Carol Davila" University of Medicine and Pharmacy, Faculty of Medicine, Parasitology Unit, Bucharest, Romania
[2]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Cluj-Napoca, Romania
[3]University of Agronomical Sciences et Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Bucharest, Romania
[4]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy
[5]Federal Institute for Risk Assessment, Berlin, Germany
[6]Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark
[7]Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, MAISONS-ALFORT cedex, France

Herbivorous animals are usually, by virtue of their diet, outside the major transmission cycles of *Trichinella* spp. However, in the last 32 years, the horse appeared as a novel vector of *Trichinella* spp. infection to humans as 15 outbreaks associated with the ingestion of horsemeat were documented in France and Italy. Romania, one of the main country exporting horses into the European Union, experienced a dramatic increase of *Trichinella* sp. infections in both domestic pigs and humans in the 1990's. Some *Trichinella spiralis* - infected horses were exported into the EU during this period. The aim of this study was to evaluate the prevalence of *Trichinella* sp. infections in horses from various Romanian regions with indirect and direct tests. In 2001 of the 3,000 serum samples tested, none resulted positive by ELISA using three different antigens (crude; excretory/secretory, ES; and stg-BSA antigens). In 2002, of the 2,992 serum samples tested, 17 (0.56%) showed in some laboratories, optical density values higher than that of the cut-off by an ELISA using ES antigens and only one was confirmed by western blot (WB). Four out of the 17 ELISA positive horses, including the horse with a confirmed serology by WB, were subjected for intensive meat examination at slaughter, but no *Trichinella* sp. larva was detected. No *Trichinella* sp. larva was detected by trichinelloscopy and artificial digestion in the 25,838 horses slaughtered in Alexandria and Timisoara between 2001-2004. Although specificity of ES ELISA seems to be high in horses during the first months after infection, serological results must be interpreted critically in term of false negative results after a long-term infection as observed in experimental studies. The

false positive results obtained by serology suggest that serological methods cannot be used yet to detect *Trichinella* sp. infection in horses. Furthermore, the lack of detection of *Trichinella* sp. infected horses by artificial digestion, suggest the very low prevalence of infection of these nematodes in horses of Romania.

Work founded in part by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156) and in part by the EU project MedVetNet (Workpackages TRICHINET and TRICHIMED).

Radu Blaga, DVM, PhD
University of Agricultural Sciences and Veterinary Medicine, Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort, France
Department of Parasitology Unit, Faculty of Veterinary Medicine
3-5 CALEA MANASTUR
400372 CLUJ-NAPOCA ROMANIA
Phone: 0040264596384
e-mail: blecgec@yahoo.com

65. Monitoring of the prevalence of *Trichinella* sp. in Swiss carnivorous wildlife

Frey, C.[1], V. Eidam[1], R. Graf[1], Ch. Wittwer[1], M.E. Schuppers[2], B. Gottstein[1]

[1]Vetsuisse Faculty, University of Bern, Institute of Parasitology, Bern, Switzerland
[2]SAFOSO, Bern, Switzerland

Trichinellosis is a zoonotic disease that is caused by different species of the nematode genus *Trichinella*. *Trichinella* spp. maintain their life cyle in many carnivorous and omnivorous animal species. So far, 11 different subspecies or genotypes have been recognized. In Switzerland, for the past decades *Trichinella* has only been found in carnivorous wildlife, such as foxes, lynxes and wolves. All *Trichinella* isolates assessed by species-specific PCR yielded *T. britovi* as causing species. Although lynxes and wolves have become extinct during the last century in Switzerland, their actual protection status allowed the re-appearance of these animal species either due to anthropogenic reintroduction (lynx) or upon natural immigration (wolf). Their population numbers are still very low. For the past decades, every dead lynx or wolf brought to pathology has also been examined for the presence of *Trichinella* sp. Thus, 15 out of 55 lynxes examined between 1999 and 2007 (prevalence 27%) were found to be positive for *Trichinella*, as tested with the artificial digestion method. From two wolves tested, one was positive for *Trichinella*. All *Trichinella*larvae recovered from lynxes and wolves were identified as *T. britovi* by PCR. The fox is recognized as the most important reservoir host of *T. britovi* in Switzerland. In the past 20 years, the fox population has experienced a four-fold increase. A study carried out in 1992, in which 538 foxes had been examined, revealed a prevalence 1.3% with regard to *Trichinella* larvae detectability in foxes. In order to assess the present infection status of the Swiss fox population, 1,400 foxes hunted or found dead all over Switzerland are presently being investigated for the presence of *Trichinella* sp. muscle stage larvae, including a subsequent molecular typing of recovered isolates.

Caroline Frey, DVM
University of Bern
Institute of Parasitology
Länggassstrasse 122
3012 Bern
Switzerland
Phone: +41 31 631 24 75
e-mail: caroline.frey@ipa.unibe.ch

66.

Survey of trichinellosis in UK wildlife

Zimmer, Irene-A[1], S.J. Hunter[1], Valerie D. Boughtflower[1], C.P. Morgan1, Keith R. Hunt[2], G.C. Smith[2], S.A. Fee[3], D.Abernethy[4], M.A. Taylor[4]

[1]Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK
[2]Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB
[3]The Agri-Food and Biosciences Institute (AFBI), Veterinary Sciences Division, 43 Beltany Road, Omagh, Co Tyrone BT78 5NF, Northern Ireland, UK
[4]Department of Agriculture and Rural Development, Dundonald House, Upper, Newtownards Road, Belfast BT4 3SB, Northern Ireland, UK

No wildlife or indigenously acquired human cases of *Trichinella* spp. have been reported in mainland Britain since 1957 and the last reports of infected pigs found in Northern Ireland date back to 1975 and 1979. From 2006, Trichinella testing of meat for human consumption is mandatory in all Member States and approved test methods are laid out in EU regulations. EU approved diagnostic methods for wildlife testing have not yet been implemented and work on harmonising wildlife testing is ongoing. In this study, an EU approved *Trichinella* testing method for pig meat was adopted, modified and validated for testing wildlife. The method has been applied to a UK wildlife surveillance programme, which has been carried out at CSL since 2004 in support of an application to officially recognise Great Britain as a region where the risk of *Trichinella* in domestic pigs is negligible. To date, all fox samples from Great Britain have been negative. With over 3000 foxes sampled since 1999, the upper 95%ile confidence limit for national prevalence of *Trichinella* is below 0.1%. We also present the first finding of Trichinella spiralis in a red fox in Northern Ireland for over 25 years.

Irene-A. Zimmer, PhD
Central Science Laboratory (CSL)
Department of Veterinary Surveillance
Sand Hutton, York
YO41 1LZ United Kingdom
Phone: +44 (0)1904 462492
(e)mail: i.zimmer@csl.gov.uk

67. An ulcerative skin lesion associated with natural *Trichinella* infection in a cat

Airas, Niina, S. Saari, S. Sankari, V. Vihma, A. Sukura

Although experimental and natural *Trichinella* spp. infections have been described, clinical manifestations are extremely rare in cats. An 8-year-old neutered male domestic shorthair cat was presented with a crusting ulcer measuring 7 mm to 4 mm at the lower left eyelid. The animal was not responding to systemic or local treatment with antibiotics. The lesion was excised and submitted for histopathology. ELISA was used to determine host-produced antibodies against *Trichinellae* in serum.

Histopathologically, the epidermis was ulcerated. The dermis was affected by a reaction consisting of interlacing bundles of fibroblasts with a mixed inflammatory infiltrate. Perivascular lymphocytic infiltration with few eosinophils was present at the peripheray of the lesion. In the center of the lesion there was a well preserved *Trichinella* larva within elliptical cyst with a collagen capsule.

ELISA showed increased serum levels of Trichinella antibodies.

The larva was identified at the genus level from the biopsy based on the location (within muscle fiber) and morphology (nurse cell formation, measurements, and presence of stichosome). The cystic capsule, intensive inflammatory reaction, and the shape of the cyst were indicative of *T.nativa*, which is the most common *Trichinella* species in Finnish wildlife. The occurrence of the larva in this lesion could have been purely accidental. However, unsuccessful treatments with antibiotics and the intralesional location of the larva are suggestive of the participation of *Trichinella* in the pathogenesis of this lesion. This is the first documented case of clinical trichinellosis in a cat with an associated ulcerative cutaneous lesion as the main clinical manifestation.

Niina Airas, DVM
University of Helsinki
Department of Basic Veterinary Sciences
P.O.Box 66
00014 University of Helsinki Finland
Phone: +358 9 191 57188
e-mail: niina.airas@helsinki.fi

68.

Trichinella pseudospiralis in swine

Beck R.[1], Beck A.[1], Lučinger S.[1], Čurković S.[1], Marinculić A.[1]

[1]Faculty of Veterinary Medicine University of Zagreb

Trichinella spiralis and Trichinela britovi are the species that were frequently found in domestic pigs and various sylvatic animals in Croatia. During routine trichinoscopy , non- encapsulated larvae were detected in the muscle tissue of the domestic swine. Artifical digestion revealed 602 muscle larvae. The tissue section analysis confirmed the presence of larvae that were not surrounded by the ordinary capsule too. Multiplex PCR identified the larvae as Trichinella pseudospiralis. It should be noted that local veterinary inspector reported the findings of strange nonencapsulated Trichinella even four times during last two years. It has to be stressed that this the first record of T. pseudospiralis in Croatia and one of very few cases of Trichinella pseudospiralis infection in domestic pigs. The detection of nonencapsulated larvae stress the need of prompt implementation of artificial digestion instead of trichinoscopy .

69. Distribution of *Trichinella spiralis* and *Trichinella britovi* in wildlife of the European Union

Pozio, E.[1], Laura Rinaldi[2], G. Marucci[1], E. Musella[2], G. la Rosa[1], G. Cringoli[2]

[1]Istituto Superiore di Sanita, Department of Infectious, Parasitic and Immunomedited Diseases, Rome, Italy
[2]Veterinary Faculty, University of Naples, Naples, Italy

Trichinella spiralis and *Trichinella britovi* are the two most important species of the genus *Trichinella* circulating in Europe. The aim of this work was to identify the habitat characteristics and environmental conditions favouring the transmission of these two pathogens in Europe, and to establish a map of risk on the occurrence of *Trichinella* spp. infection in wildlife. The current available information on *Trichinella* spp. isolates from Europe, originates from the database of the International *Trichinella* Reference Centre. Of 1,482 European isolates, 535 belonged to *T. spiralis* and 706 to *T. britovi*. A Geographical Information System (GIS) of Europe was constructed utilizing data layers on land cover and elevation above sea level (asl). All the geo-referenced reports of *T. spiralis* and *T. britovi* detected in the red fox (*Vulpes vulpes*) and in the wild boar (*Sus scrofa*) were inserted into the GIS. A strong association between *T. spiralis* and the wild boar and between *T. britovi* and the red fox was observed. In Germany, Poland and Spain, *T. spiralis* was prevalent (53-87%) in the red fox and/or wild boar populations than *T. britovi* (13-47%), whereas in Bulgaria, Estonia, France, Italy, Latvia, Lithuania, The Netherlands, Romania and Slovak Republic *T. britovi* (73-100%) was prevalent than *T. spiralis* (0-27%). According to the elevation, *T. britovi* circulates at a higher elevation than *T. spiralis* ($P<0.0001$) and the elevation trend is statistically significant ($P<0.0001$) in Central-North Europe, suggesting the higher susceptibility of *T. spiralis* larvae to lower temperature than that of *T. britovi*. Both *T. spiralis* and *T. britovi* circulate in forest and seminatural areas and in agricultural areas, whereas their circulation in domestic areas is negligible.

Edoardo Pozio, PhD
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299, 00161Rome
Italy
Phone: +39 06 4990 2304
e-mail: edoardo.pozio@iss.it
Web: www.iss.it/crlp/

70. Trichinellosis in wild fauna (synanthropic and sylvatic) from Argentina

Ribicich, M[1], J. .Bolpe[2], E. Scialffa[2], N. Cardillo[1], P. Diaz Perez[1], A. Betti[1], F. Tellechea[1], M.L. Cambiaggi Holzman[1], A. Rosa[1]

[1]Facultad de Ciencias Veterinarias. UBA
[2]Ministerio de Salud, División de Zoonosis Rurales, Provincia de Buenos Aires

In recent years, natural infection with *Trichinella* has been described in more than 150 mammalians species. Argentina has few reports involving *Trichinella* infection in wild animals. In this study, muscle tissue was obtained from wild animals with the aim of evaluating the presence of *Trichinella*. A total of 115 muscles samples were collected from wild animals and these samples were used to determine the presence of *Trichinella* larvae by artificial digestion. These muscle samples of 12 wild animal species belonging to: 14 opossums (*Didelphis albiventris*), 6 armadillos (*Chaetophractus villosus*), 9 capybaras (*Hydrocaeris hydrocaeris*), 1 puma (*Puma concolor*), 1 grey fox (*Lycalopex gymnocercus*), 6 coypus (*Myocastor coypus*), 6 skunks (*Conepatus chinga*), 2 ferrets (*Galictis cuja*), 52 rats (*Rattus norvegicus*), 6 mice (*Mus musculus*), 9 wild boars (*Sus scrofa*), and 3 wild cats (*Felis geoffroyi*). *Trichinella* infection was detected in 1 puma, with 2 larvae per 1 gram (LPG), 1 wild boar, with 420 LPG and 9 rats with a range between 0.1 to 54.8 LPG. The parasite had been identified not only in synanthropic animals (*Rattus norvegicus*) with a high prevalence (17.3%), but also in sylvatic fauna (wild boar and puma) with a prevalence of 3.17%. The presence of *Trichinella* infection among wild animal populations suggests a wild cycle of transmission in Argentina with the risk to be as a reservoir for humans and domestic animal. The evidence of high prevalence in rats emphasize the needs of improve pigs breeding mainly in small individual farms without adequate technology, quality feeds and veterinary services.

Mabel Ribicich, Dra, Professor
Faculty of Veterinary University of Buenos Aires
Department of Parasitology and Parasitic Diseases
Chorroarin 280
1427 CWO Argentina
Phone: 54 11 4580 2820
e-mail: mribicich@fvet.uba.ar

71.

Infection with *Trichinella* spp. in seals from Arctic waters

Møller L.N.[1], E. Petersen[2], A. Koch[3], M.Melbye[3], M.O. Christian[1], C.M.O. Kapel[1]

[1]Institute of Ecology, Faculty of Life Sciences, Copenhagen University, Frederiksberg, Denmark.
[2]Department of Infectious Diseases, Aarhus University Hospital, Aarhus.
[3]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.

Introduction: In Greenland, previous studies have reported *Trichinella* in (*Phoca hispida*) (0.06%) bearded seals (*Erignathus barbatus*) (0.8%). An experimental study has demonstrated that grey seals (*Halichoerus grypus*) are highly susceptible to infection with *Trichinella native*. The present study aimed to determine the prevalence of *Trichinella* in the four seal species hunted and eaten by Greenlandic Inuit: ringed seal (*Phoca hispida*), harp seal (*Phoca groenlandica*), hooded seal (*Cystophora cristata*), and bearded seal (*Erignathus barbatus*) and to type any recovered specimens by molecular tools. Additionally, a serological assay was evaluated on the material.

Methods: Muscle samples from four seal species, hooded seal (*Cystophora cristata*), harp seal (*Phoca groenlandica*), ringed seal (*Phoca hispida*), and bearded seal (*Erignathus barbatus*) were collected from Greenland between 1982 and 2004. Muscle juices from the samples were tested by ELISA using two different antigens to detect *Trichinella* antibodies in muscle fluid, and by HCl-pepsin digestion to detect muscle larvae. Positive samples from the ELISA were examined further by Western blot.

Results: *Trichinella* larvae were recovered from a total of six seals from two species; 0.16% of ringed seals and 2.3% of hooded seals. All muscle larvae recovered were examined by multiplex-PCR. In general, the DNA of the recovered larvae was poorly preserved due to the long storage times of the muscle samples. One sample from a ringed seal confirmed infection with *Trichinella nativa*. The serological examination of the muscle fluid samples detected positives among ringed seals, harp seals, and hooded seals.

Conclusion: The low grade infection in the seals most likely only pose a limited risk for clinical disease, but may explain a high level of sero-positivity in the native population consuming seal meat.

72. Trichinellosis in wolves in Croatia

Beck A.[1], Beck R.[1], Kusak J.[1], Lučinger S.[1], Mihaljević Ž.[1], Živičnjak T.[1], Huber, Đ.[1], Gudan, A.[1], Pozio E.[2], Marinculić A.[1]

[1]Faculty of Veterinary Medicine University of Zagreb, Zagreb
[2]Istituto Superiore di Sanita, Rome, Italy

The aim of the present study was to investigate the prevalence of *Trichinella* infection in wolves (Canis lupus) permanently present on 17468 km2 with individuals. Muscle samples were collected from 62 wolves killed between 1996. and 2007. and analysed by artificial digestion. The muscle larvae were detected in 18 wolves (29, 32%) and also typed by multiplex PCR. *Trichinella britovi* was the predominant specie. Curiously *T. spiralis* was also detected. The presence of so called domestic *Trichinella* was a real suprise especially because till now in this non - endemic region only *T. britovi* was detected in wild animals. Infection rate was from 0.3 to 45.9 larvae per gram. Geographical distribution of infected animals varies. No infected animals were found in the region of Gorski Kotar which has the same altitude over the see level as the region of Lika were almost all wolves were found infected. Interestingly, infected wolves were also found in Dalmatia.

73.

Trichinellosis in wild boars in Croatia

Florjančić T.[1], Beck R.[2], Tončić J.[1], Martinković F.[2] Živičnjak T.[2], Lučinger S.[2], Antunović, B.[1], Gašpar A.[3], Bošković I.[1], Marinculić A.[2], Gross-Bošković A.[4], Dragičević P., Urošević B.

[1]Agronomy Faculty University of Osijek
[2]Faculty of Veterinary Medicine University of Zagreb
[3]Ministry of Agriculture, Forestry and Water Resources
[4]Croatian Food Agency

In order to study the epidemiology of trichinellosis with special emphasis on wild boars a large scale study was performed. The goals of the study were to define the prevalence of trichinellosis in wild boars, to determine the etiological agents and to evaluate the use of ELISA in epidemiological investigation. Totally 1498 diaphragms were collected during three years period from 1498 (1003 diaphragms during the first collection period, 495 diaphragms during the second collection period) Isolated *Trichinella* larvae were also analysed by multiplex PCR in order to understand possible transmission between domestic pigs and wild boars. The prevalence in wild boars among the first study, mainly from non-endemic region was less than 0, 1% while among the second study the prevalence was higher (1, 6%). Studies on genotypes suggest that *Trichinella britovi* is the most common etiological agent in wild boars found in non endemic region while *T. spiralis* and *T. britovi* were common in the endemic region. The presence of *T. spiralis* in wild boars presented mostly in the domestic habitat suggesting a link between the sylvatic and domestic cycle. Another important conclusion was the comparison between the accuracy of results of ELISA with Western Blotting and the real muscle larvae burden after artificial digestion.

74. Biochemical, haematological and serological changes in experimental infestation with *Trichinella britovi* in pig

Oltean, Miruna, Adriana Titilincu, A. Fenesan, V. Cozma

Several biochemical and haematological changes are currently observed during *Trichinella* infection in humans and animals. The aim of the present work was to detect biochemical, haematological and serological changes occuring during *Trichinella britovi* infection in pig. 5 pigs were infested with 15.000 larvae, respectively 1.500 larvae. Blood samples were recovered individually for up to 80 days post-infection (p.i.). Increased values were revealed for ASAT, ALAT, CPK and eosinophilis. No changes were identified for LDH, PA, Creatinine, haematies, haematocrite, haemoglobin. Serum antibodies identified by ELISA ES (Pourquier Institute) were revealed from 10 days p.i., for the group infected with 15.000 larvae (3/5 pigs reacted positively). By day 26 p.i. the entire group presented higher values than the cut-off. Furthermore, the pigs infested with 1.500 larvae were detected positive for antibodies anti-*Trichinella* begining with day 40 post infection.

Work founded by the Romanian Education Ministry thru the "CEEX" project (contract 99-2006-2008).

Adriana Titilincu, DVM, PhD.
University of Agricultural Sciences et Veterinary Medicine CLUJ NAPOCA, Faculty of Veterinary Medicine
Department Parasitology Unit
calea Manastur nr 3-5
Post Code: 400372 Cluj-Napoca Romania
Phone: 0040264596384
e-mail: atitilincu@yahoo.co.uk

75. *Trichinella nelsoni* and *Trichinella* T8 co-infection in a lion (*Panthera leo*) of the Kruger National Park (South Africa)

Marucci G.[1], L.J. La Grange[2], G. La Rosa[1], E. Pozio[1]

[1]Istituto Superiore di Sanita, Department of Infectious, Parasitic and Immunomedited Diseases, Rome, Italy
[2]Vet Services, Dep Agriculture and Land Administration, Nelspruit, S. Africa

Trichinella sp. was discovered in wildlife of the Kruger National Park (KNP) of South Africa since 1967. More recently in the KNP, *Trichinella* T8 was detected in a spotted hyena (*Crocuta crocuta*) and in a lion (*Panthera leo*), and *Trichinella nelsoni* in another lion, suggesting the circulation of two distinct taxonomic entities in this area. The discovery of this sympatry has opened a debate on the distribution area of the two taxonomic entities and on the existence of a gene flow between them. In March 2007, a 15 year old lion was killed in the Mthethomusha Nature Reserve bordering the south-western border of the KNP, because it caught domestic livestock near a communal diptank at Luphisi. Encapsulated larvae of *Trichinella* sp. were observed in the muscles by trichinoscopy. Larvae were isolated from the muscles by artificial digestion: 41 larvae/g in the tongue, 25 larvae/g in the masseter and 16 larvae/g in the pterygoides. The molecular identification of the single *Trichinella* sp. larvae by multiplex PCR, revealed the presence of a mixed infection with both *T. nelsoni* and *Trichinella* T8. No larva with an hybrid pattern between the two genotypes was observed. A pool of larvae of both genotypes was injected per os in mice. No hybrid larvae were detected two months later. This is the first time that a mixed infection with two *Trichinella* genotypes has been detected in a host from the KNP. These results show the KNP as a region, where both *Trichinella* T8 and *T. nelsoni* circulate among wildlife and, despite the sympatry status, natural and laboratory data seem to exclude the possibility of a gene flow between these two genotypes, confirming their evolutive separation.

Gianluca Marucci, PhD
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299
00161Rome, Italy
Phone: +39 06 4990 2310
e-mail: gianluca.marucci@iss.it
Web: www.iss.it/crlp/

76. Preliminary results on *Trichinella spiralis* infection in wild boars (*Sus scrofa*) from a protected area of Argentina

Maris Venturiello, Stella[1], Melina Cohen[1], L.X. Barrios Castro[1], G.G. Nuńez[2], G.A. Blanco[2], E. Pozio[2] , Susana N. Costantino[1]

[1]El Palmar, National Park, Entre Rios, Argentina
[2]Isituto Superiore di Sanita, Rome, Italy

Trichinella spiralis is a widespread zoonotic pathogen in domestic pigs and synanthropic animals living in Argentina; however, very few information is available on the circulation of *Trichinella* spp. among wildlife of Argentina. The aims of the work were: A) to detect *Trichinella* spp. larvae in wild boars (*Sus scrofa*) of a protected natural park located in an area of the Argentina which had been considered *Trichinella*-free; B) to identify the *Trichinella* species circulating in the area by a PCR-derived method; and C) to detect the exact locality where infected and non-infected animals are circulating by the Geographical Information System. Muscle samples (76ą21g) from 54 wild boars killed by hunters, were analysed individually by the artificial digestion method. *Trichinella* spp. muscle larvae were detected in 5 (9.2%) of wild boars, with a parasite burden ranging from 0.01 to 0.34 larvae/g. Muscle larvae collected from a positive wild boar have been identified as belonging to *T. spiralis*. The geographical distribution pattern of the infected animals did not show any correlation with human settlements. Nevertheless, the origin of the infection from the domestic cycle cannot be ruled out. These results show the circulation of *T. spiralis* in wild animals living in their natural habitat and, at the same time, how a lack of reports on *Trichinella* spp. infection in domestic animals and humans, in the absence of surveys, does not allow to consider an area as *Trichinella*-free.

Stella Maris Venturiello, Ph.D.
Chair of Immunology, Faculty of Pharmacy and Biochemistry,
University of Buenos Aires. IDEHU-CONICET
Department of Microbiology, Immunology and Biotechnology
Junin 956; 1113 Buenos Aires
Argentina
Phone: 54-011-4964-8259/8260
e-mail: sventuri@ffyb.uba.ar

77. Trichinellosis in humans: comparison of different ELISA procedures for diagnosis

Moskwa, Božena[1], Justyna Bien[1], W. Cabaj[1], Elžbieta Kacprzak[2], J. Stefaniak[2], B. Koudela[3]

[1]Witold Stefanski Institute of Parasitology of the Polish Academy of Sciences, Twarda Street 51/55, 00-818 Warsaw, Poland
[2]Clinic of Parasitic and Tropical Diseases, Poznan University of Medical Sciences, Fredry Street 10, 61-701 Poznan, Poland
[3]Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno, Palackého 1-3, 612 42 Brno, Czech Republic

The quality of available serological methods to detect *Trichinella*-specific antibodies is out of almost importance for epidemiological analysis. Trichinellosis is a parasitic disease acquired by humans after ingestion of *Trichinella spiralis* infected meat of somestic pigs or wild animals. This disease is still a significant problem for public health in Poland. Trichinellosis can be deceived with several other diseases because similar clinical symptoms are developed by infected patients.

The present study compare the usefulness of the results obtained by three ELISA procedures for *Trichinella* spp. diagnosis in human outbreaks. Serum samples were obtained from 52 symptomatical patients from 3 human outbreaks.

The main differences in ELISA procedures were: the protein concentration in antigen, dilution of human serum samples and the time of conjugate incubation. Additional differences were noticed, for eg. ES antigen preparation procedures and kind of *T. spiralis* isolate used in these procedures.

The results revealed that the differences in procedures have influenced ELISA results in cut off values and different positivity rates for individual outbreak. The seroprevalence of trichinellosis in examined outbreaks oscillated between 8.0 and 81.2 % depending on the outbreaks and the serological technique used.

Althought the ELISA is used for human trichinellosis since than 25 years, to clarify the results, the standardization of procedures is needed.

Bozena Moskwa, PhD
Witold Stefanski Instiute of Parasitology
Polish Academy of Sciences
51/55 Twarda Street
00-818 Warsaw
Poland
Phone: + 48 22 620 62 26
e-mail: moskwa@twarda.pan.pl
Web: iparpas@twarda.pan.pl

78. Evaluation of the vertical transmission of *Trichinella spiralis* during human pregnancy

Nuńez, G.G., Susana N. Costantino, Teresa Gentile, Diana Stivale, Stella Maris Venturiello

The clinical background, vertical transmission of *Trichinella spiralis*, and the helmithotoxic activity of sera were evaluated in 4 cases of infected pregnant women. Parasitological and immunological parameters were analyzed. Patients presented a clinical frame and a seroconversion pattern typical of a *Trichinella* infection independently of the gestation trimester. No secondary manifestations were found. Patients gave birth to healthy infants on full term even the one treated with mebendazole. Studies performed in placentae and umbilical cords by artificial digestion and/or immunofluorescence did not reveal the presence of neither the parasite nor its antigens (Ags). Anti-ML-ESP (IgG, IgE, IgA and IgM) were found in maternal sera. Specific IgG, IgE and IgA were found in the umbilical cord sera and only one of them had specific IgM as immune-complex. In the latter infant circulating parasite Ags were also found by ELISA and WB even after 10 months after birth. Sera from the patients were able to induce the NBL death in in vitro cytotoxicity assays, even in the absence of specific antibodies, this effect was abrogated by mefipristone (31.0+/-8.7%vs9.7+/-8.2%). Our results suggest that in human trichinellosis during pregnancy there is an enhanced helminthotoxic status towards the NBL, dependent upon the progesterone leading to a mild or moderate course of the infection. However, the transplacentary passage of migrant larvae to the foetus has occurred.

Stella Maris Venturiello, Ph.D., Proffessor,
Chair of Immunology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires-Humoral Studies Institute (IDEHU-CONICET)
Department of Microbiology, Immunology and Biotechnology
Junin 956 4ş floor
1113 Buenos Aires Argentina
Phone: 54-011-4964-8259/60
e-mail: sventuri@ffyb.uba.ar

79. Evaluation of the ELISA-Western blot test on serially collected serum samples for the diagnosis of human trichinellosis

Krivokapich S., Viviana Molina, Graciana Ayesa, Marcela Monfellano, Patricia Arbusti, E. Guarnera

Trichinellosis is an parasitic zoonosis widely distributed in Argentina. In the present work, we performed a serological assessment of patients suspected of being recently infected with *Trichinella*, coming from different endemic areas of Argentina, during years 2004 and 2005. The analysis was conducted with a combined ELISA and Western blot assay of serially collected serum samples. ELISA was applied as a screening analysis and Western blot as a confirmatory test, using excretory-secretory antigens of the muscle larvae of *T. spiralis*. From a total number of 636 patients, 264 (41.6%) returned to have a second sample, 15 days later, and 97 (15,3%) completed the schedule of the third sample, obtained 30 days from the previous one. In the second and third testing rounds, only were included the samples that were negative in the previous assay. The results from this serological analysis show the detection of 23.3%, 45.1% and 22.6% of positives in the first, second and third samples, respectively. These data indicate that the ELISA-Western blot assay using serial serum samples assures more accurate diagnosis of Trichinellosis, because it allows detection of positive samples that otherwise by analyzing just one sample would have been false negatives.

Silvio Krivokapich, Licenciado en Genética (Geneticist)
INEI, ANLIS, Dr. Carlos G. Malbrán
Department of Parasitology, Laboratory of Trichinella and Trichinellosis
Av. Velez Sarsfield 563,
1281 Buenos Aires Argentina
Phone: 54-11-4031-7437
e-mail: silkri@anlis.gov.ar

80.

Dose response of *Trichinella* from human outbreak data

van der Giessen, J.[1], Katsuhisa Takumi[1], M. Fonville[1], P. Teunis[2]

[1]National institute of Public Health and the Environment, Department of LZO
[2]**Dept. IMA, National Institute of Public Health and the Environment, Bilthoven, Netherlands**

The study was supported by the 6th Research Framework Programme of the European Commission within the Network of Excellence "Med-Vet-Net" (WP TrichiMed).

Joke van der Giessen, PhD.
National institute of Public Health and the Environment
Department of LZO
Antonie van Leeuwenhoeklaan 9/P.O.Box 1
3721 MA Bilthoven Netherlands
Phone: +313 02 74 39 26
e-mail: joke.van.der.giessen@rivm.nl

A theoretical dose response model was developed for a parasite that needs to sexually reproduce in the small intestines. The model is based on the assumption that infection can occur if and only if one or more female parasites and one or more male parasites survive in the host intestine. Using the model we analysed the dose response from six outbreaks of *Trichinella* that reported the number of individuals who consumed the contaminated meat, the number of infected individuals, the number of the parasites per gram of the meat, and the amount in gram of the meat consumed. The parasite species were pooled for the analysis. The sex ratio of female parasites is assumed to be 70%. The analysis indicated that the likelihood of human trichinellosis is substantial even when the dose of *Trichinella* is very low, and suggests that a safety guideline based on a concept of minimal infectious doses needs to be revised.

81. The birth of a *Trichinella britovi* focus in the Mediterranean island of Sardinia (Italy)

Pozio E.[1], P. Cossu[2], G. Marucci[1], M. Amati[1], Alessandra Ludovisi[1], Maria Angeles Gomez Morales[1], G. La Rosa[1], T. Firinu[2]

[1]Istituto Superiore di Sanita, Department of Infectious, Parasitic and Immunomedited Diseases, Rome, Italy
[2]Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

The islands of the Mediterranean basin were considered *Trichinella*-free for the lack of reports of *Trichinella* sp. infections in animals and humans in the last 60 years. Between 2005 and 2007, three outbreaks of human trichinellosis involving 11, 8 and 1 persons, occurred in the villages of Orgosolo and Lanusei (Nuoro province, Sardinia island). All the infected persons have consumed row pork from three pigs free-ranging in the surrounding mountain area of Orgosolo. Between January and March, 2006, muscle samples from 681 swine (325 free-ranging, 356 breed in small corrals) were tested by artificial digestion. *Trichinella* sp. larvae were detected in four sows (1.2%) of the free-ranging group. All larvae, including those from the sausages, which were the cause of the human infections, were identified as *Trichinella britovi*. All infected pigs originated from the same area of the Orgosolo municipality. The epidemiological investigation revealed that the two sows of 2 years of age had acquired the infection for the consumption of scraps from the sow, which was the source of infection of the first human outbreak. In the winter of 2006, 6,188 wild boars (*Sus scrofa*) (129 of which from the Orgosolo municipality) and 13 red foxes (*Vulpes vulpes*) killed by hunters in the island resulted negative for *Trichinella* sp., suggesting that this parasite was still restricted to free-ranging pigs. In 2004, a focus of *T. britovi* was discovered among free-ranging pigs of a remote area of the island of Corsica (France). The origin of these two foci is unknown but deserves further investigations.

Edoardo Pozio, PhD
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299
00161 Rome, Italy
Phone: +39 06 4990 2304
e-mail: edoardo.pozio@iss.it
Web: www.iss.it/crlp/

82. Review of recent outbreaks of trichinellosis due to the consumption of meat from Canadian black bears

GajadharA., L. Forbes

Canadian Food Inspection Agency,
Centre for Food-borne and Animal Parasitology,
Saskatoon Laboratory, Canada

Since 2000, there have been three major outbreaks of trichinellosis due to the consumption of meat of black bears infected with *Trichinella nativa*. In the first outbreak, bear meat boiled for 2-4 hours or smoked/dried for 2-3 days was consumed by 78 individuals in northern Saskatchewan. Confirmed cases consisted of classic myopathic symptoms and positive ELISA results. *Trichinella* larvae recovered from different parts of the bear's musculature ranged from 0 to 310 larvae per gram (LPG). Another outbreak involving 42 individuals occurred in 2005 in Vancouver. Bear meat previously frozen for at least 3 days was barbecued, fried or stewed and consumed at 3 separate events. Clinical symptoms and serology were used to confirm cases of trichinellosis, and digestion assay of the bear's leg muscle revealed over 300 LPG. Also in 2005, meat from a black bear in northern Quebec was the source of an outbreak of trichinellosis in France. Fresh or previously frozen meat was consumed by 10 hunters and 15 acquaintances, and 17 cases of trichinellosis were confirmed. *Trichinella* larvae were recovered from the bear meat and muscle biopsies of patients. Multiplex PCR assay performed at the Canadian Centre for Foodborne and Animal Parasitology identified the etiological agent from all three outbreaks as *Trichinella* nativa.

Alvin Gajadhar, PhD
Canadian Food Inspection Agency
Centre for Foodborne & Animal Parasites
116 Veterinary Road, S7N 2R3 Saskatoon
Canada
Phone: 1-306-975-5344
e-mail: agajadhar@inspection.gc.ca

83.

Epidemiology of trichinellosis in Germany from 1996-2006

Jansen, A.[1], Irene Schöneberg[1], K. Stark[1], Karsten Noeckler[2]

[1]Robert Koch Institute, Dept. for Infectious Disease Epidemiology, Seestrasse 10 D-13353 Berlin, Germany
[2]Federal Institute for Risk Assessment, Diedersdorfer Weg 1, D-12277 Berlin, Germany

Although trichinellosis is considered to be under control in Germany, a significant number of cases continue to occur. Our objective was to investigate the characteristic of laboratory-confirmed cases of trichinellosis in Germany over the last decade in order to provide an up-to-date epidemiologic profile of the disease, which may serve as a suitable basis for targeted public health measures.
Data on demographics, courses of disease, and sources of infection were compiled from national surveillance data (1996-2006) and from a questionnaire-based survey (1996-2000). The Mann-Whitney test or the student-t test were used for comparative analysis of continuous variables, as appropriate. Incidence rate ratios (IRR) and 95% confidence intervals (95%CI) were calculated. A p-value <0.05 was considered as significant. From 1996-2006, 95 laboratory-confirmed infections and 12 outbreaks were reported. Highest incidence was found in immigrants from southeast European countries (0.03/100,000 vs. 0.01/100,000 in the German population) with an incidence rate ratio of 26.0 (95%CI 11.6-51.8). Mean diagnostic delay was 49 days. Among patients with reported source of infection (85%, n=81), the consumption of pork (mostly originating from endemic countries) represented the most frequent exposure (93%; n=75). In addition, trichinellosis cases were associated with both indigenous (n=1) and imported (n=5) wild boar meat. Although domestic pigs in Germany are practically free from *Trichinella* spp., the parasite is found in 0.003% of German wild boars. Trichinellosis still remains a public health issue in Germany, especially among individuals with a migrational background. Immigrants from endemic countries need to be educated about the risks of consuming raw or inadequately cooked pork and wild boar products from their homelands. In addition, German health care providers need to be aware of trichinellosis, especially in areas with a large immigrant population.

Andreas Jansen, PhD
Robert Koch Institute
Department for Infectious Disease Epidemiology
Seestrasse 10 D-13353 Berlin, Germany

84. Human trichinellosis in Bulgaria – epidemiological situation and trends

Kurdova-Mintcheva Rossitza, Marinella Ivanova, Diana Jordanova

National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

Trichinellosis is a major food-borne zoonosis in Bulgaria with a high health, social and economic impact. Every year epidemic outbreaks and sporadic cases are registered in the country. The aim of the study was to analyze collected at NCIPD official data of standard protocols for epidemiological surveillance and control of human trichinellosis applied in Bulgaria, and to estimate the epidemiological trends during the past 17 years (1990-2006). Since 1991 human trichinellosis has become a re-emerging zoonosis in Bulgaria. During 1990-2006 a total number of 148 trichinellosis outbreaks, each of them involving about 2–200 patients were registered. Altogether 7268 persons have consumed infected with Trichinella larvae meat or meat products, among them – 2092 with symptomatology and two with lethal outcome. The epidemiological and clinical survey carried out revealed that 57 of the outbreaks were caused by consumption of insufficiently cooked meat and meat products of wild boar, 70 – of pork, 4 of products prepared of both pork and wild boar meat, and in 17 – the source of infection was unidentified. The peaks of outbreaks (12 per year) were registered in 1994, 1998, 2002 and 2003. In the past three years there is a trend of decreasing of outbreaks number (2004 – 6, 2005 – 3 and 2006 – 7) morbidity rate. A circulation of two Trichinella species - *T. spiralis* and *T. britovi* in the domestic and sylvatic cycles of trichinellosis was established in Bulgaria.

Rossitza Kurdova-Mintcheva
National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria
Department of Parasitology and Tropical Medicine
26, Yanko Sakazov Blvd, 1504 Sofia
Bulgaria
Phone: +35928438002
e-mail: kurdova@ncipd.org
Web: www.ncipd.org

85. The emergence of trichinellosis in Romania: a history of food habits

Blaga, R.[1], B. Durand[1], V. Cozma[2], P. Boireau[1]

[1]Epidemiology Unit, AFSSA, 23, avenue du Général de Gaulle BP 67– 94703 MAISONS-ALFORT cedex, France
[2]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, calea Manastur nr 3-5, 400372 Cluj-Napoca, Romania

Romania is one of the most affected countries in the world by trichinellosis with 780 humans cases out of 3109 in 2004 (ICT survey of 2004, (http://monsite.wanadoo.fr/intcomtrichinellosis/). It is probably correct to assume that trichinellosis, was present in Romania, long before the discovery of Trichinella spiralis by James Paget in 1835, but its dispersion certainly differs upon the origins of the population that is concerned. The population of Romania is composed of natives as well as Hungarians and Germans, migrated during the 10th and 12th century, the former two populations being situated predominately in Transylvania. Using historical and early epidemiological data, we investigate whether the geographical and socio-cultural conditions have influenced the emergence of trichinellosis in Romania, since the food habits differ greatly. Results confirm the conclusions suggested by the available historical data: a strong socio-cultural determinant for the emergence of trichinellosis in Romania, through the particular food habits of German populations established for a long time in Romania. As these populations became established mainly in specific areas of Romania (of Transylvania), this socio-cultural determinant also induced the specific geographic distribution of trichinellosis, with an elevated incidence rate in Transylvania; a distribution which was still observed in the 60's. Similarly according to Hall, cited by Gould (1945), the population group which is most frequently infected in epidemics of trichinellosis in the United States, are the Germans.

This work was supported by grants of EU TRICHINET (MedVetNet) and TRICHIMED (MedVetNet).

Radu Blaga, DVM, PhD
University of Agricultural Sciences and Veterinary Medicine, Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort, France
Department of Parasitology Unit, Faculty of Veterinary Medicine
3-5 CALEA MANASTUR
400372 CLUJ-NAPOCA ROMANIA
Phone: 0040264596384
e-mail: blecgec@yahoo.com

86. A dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years

Blaga R.[1], B. Durand[2], Silvia Antoniu[3], C. Gherman[1], Carmen M Cretu[4], Carol Davila[4], V. Cozma[1], P. Boireau[5]

[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Cluj-Napoca, Romania
[2]Epidemiology Unit, AFSSA, Maisons-Alfort, France
[3]University of Agronomical Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Bucharest, Romania
[4]University of Medicine and Pharmacy, Faculty of Medicine, Parasitology Unit, Bucharest, Romania
[5]Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort, France

According to the International Commission on Trichinellosis survey in 2004, Romania had the world highest incidence of trichinellosis. Epidemiologic data for each county were collected and analyzed for two different time periods: before (1980–1989) and after (1990–2004) the end of the communist regime. Data were analyzed separately for Transylvania and the rest of the Romanian counties. During the past 25 years, 28,293 human cases of trichinellosis were reported with an incidence of 51.0 cases per 106 person-year. An important increase in the incidence was observed from 1980 to 1989 compared with the 1990–2004 period. For the entire period, the incidence rate obtained for Transylvanian counties (82.2 cases per 106 person-year) was higher than the incidence rate obtained for the other counties (35.7 cases per 106 person-year). Hypotheses and facts contributing to the heterogeneity of human trichinellosis cases are discussed.

Work founded by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156) TRICHINET/ TRICHIMED workpackage (MedVetNet).

RADU BLAGA, DVM, PhD
University of Agricultural Sciences and Veterinary Medicine, Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort, France
Department of Parasitology Unit, Faculty of Veterinary Medicine
3-5 CALEA MANASTUR
400372 CLUJ-NAPOCA ROMANIA
Phone: 0040264596384
e-mail: blecgec@yahoo.com

87. *Trichinella* infection in Greenland: temporal and spatial variation in seroprevalence in a high-risk population

Møller L.N.[1], A. Koch[2], M. Melbye[2], E. Petersen[4], S. Andersen[3], P. Laurberg[3], C M.O. Kapel[1]

[1]Institute of Ecology, Faculty of Life Sciences, Copenhagen University, Frederiksberg, Denmark.
[2]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.
[3]Department of Endocrinology and Medicine, Aalborg Hospital, , Aalborg, Denmark.
[4]Department of Infectious Diseases, Aarhus University Hospital, Aarhus

Introduction: Outbreaks of human trichinellosis from consumption of game meat are frequent in Greenland. Epidemiological studies have been scarce and this study aimed to compare the presence of *Trichinella* antibodies in historical and recent human blood sera to evaluate temporal and spatial variations.
Methods: Human blood samples were collected from Nuuk, Upernavik, Qaanaaq, and Ammassalik municipalities during two periods, 1979-1981 and 1998-2004. Samples were initially screened for Trichinella-specific IgG antibodies by ELISA and by immunoblot as a confirmatory test. To assess the background exposure level of Trichinella infection in game from different parts of Greenland, muscle tissue from sled dogs, which are generally fed offal from game, were collected from Sisimiut, Ilulissat, Qaanaaq, and Ammassalik municipalities and analyzed for the presence of *Trichinella* muscle larvae.
Results: The prevalence of infected dogs gradually increased from south to north (Sisimiut (2.5%) and Ammassalik (14%) to Ilulissat (23%) and Qaanaaq (67%)). The human seroprevalence was high in communities depending on game meat consumption but decreased in all municipalities over the 20-year study period.
Conclusion: It was concluded that the temporal variation likely reflected an increase in the consumption of imported food in relation to traditional food in the southernmost larger towns, and that the geographical variation in *Trichinella* prevalence in sled dogs and humans was consistent with consumption of game meat.

C M.O. Kapel
Institute of Ecology
Faculty of Life Sciences
Copenhagen University
Frederiksberg, Denmark

88.

Transmission risk of human trichinellosis

Takumi, Katsuhisa[1], M. Fonville[1], P. Teunis[2], Isabelle Vallee[3], P. Boireau[3], K. Noeckler[4], J. van der Giessen[1]

[1]Dept. LZO, National Institute of Public Health and the Environment, Bilthoven, The Netherlands.
[2]Dept. IMA, National Institute of Public Health and the Environment, Bilthoven, The Netherlands
[3]JRU BIPAR, AFSSA LERPAZ, Maisons-Alfort, France
[4]2Federal Institute for Risk Assessment, Berlin, Germany

Trichinella is a major food-borne parasitic pathogen causing hundreds of infections in humans within Europe each year, and is listed in the EU Zoonoses Directive 2003/99/EC. Foxes and wild boars are the main animal reservoir species in Europe. Also small rodent species may be important as reservoir animals. Transmission of *Trichinella* species between wildlife and the risk it poses to humans via consumption of contaminated pork meat has not been quantified. One pathway by which human trichinellosis can occur is rat – swine – human route. To evaluate transmission risk of this pathway experimental *T. spiralis* infection was performed with doses as few as 10 parasites and the data set was analysed using a newly developed dose response model that describes lpg. Experimental *T. spiralis* infection in swine was analyzed in a similar way. Furthermore six outbreaks of human trichinellosis were analysed for dose response. Risk of human trichinellosis via rat – swine – human pathway was simulated by Monte Carlo. A pair of female and male parasites representing the lowest pressure from the environment led to the probability of human trichinellosis equal to 8% via the pathway. Therefore, low infection pressure from wildlife presents a relatively high risk of human trichinellosis via consumption of contaminated pork meat. Hundred percent of rats were infected with only 10 parasites and their high susceptibility indicates that rats are important in the transmission.

The study was supported by the 6th Research Framework Programme of the European Commission within the Network of Excellence "Med-Vet-Net" (WP TrichiMed) and the Inspectorate of Health Protection and Veterinary Public Health, Food and Consumer Product Safety Authority (VWA), the Netherlands.

Katsuhisa Takumi, PhD
Dept. LZO, National Institute of Public Health and the Environment
Antonie van Leeuwenhoeklaan 9
3721MA Bilthoven, The Netherlands

89. Heart specific antigens recognized by late trichinellosis patient sera

Bruschi, F.[1], F. Pratesi[2], Cristina Tommasi[2], J. Dupouy-Camet[3], P. Migliorini[2]

[1]Department of Experimental Pathology, M.B.I.E., Universita' di Pisa
[2]Dipartimento di Medicina interna, Universita di Pisa, Pisa, Italy
[3]Centre des reference des Trichinella Hospital Cochin, Paris Descartes, Paris, France

The aim of the present paper was to investigate the possible presence in trichinellosis patient sera of antibodies recognizing human tissues, particularly the myocardium.
Patients and Methods: 21 sera from late period trichinellosis patients were tested by immunoblot on extracts from human heart ventricle wall, spleen, placenta, kidney and skeletal muscle. Pre-absorption with *Trichinella spiralis* muscle larva crude extracts was used to remove parasite-specific antibodies.
Results: patient sera only and not normal sera recognized several antigens in human tissues such as myocardium and skeletal muscle. On human heart ventricle wall, 42% of sera reacted with a protein of 68 kDa ($p<0,05$ compared to normal sera). This reactivity was not observed on kidney, placenta and spleen extracts. However, it is not significantly different in patients with or without cardiac involvement. Moreover, very few bands are observed on these tissues as compared to heart, thus suggesting a high tissue specificity of the trichinellosis sera reactivity.
Preliminary pre-absorption experiments are in favour of a molecular mimicry mechanism.
In conclusion, this study identifies heart specific autoantibodies in trichinellosis patient sera, their role in the pathogenesis of cardiac involvement being still unclear.

Fabrizio Bruschi, Professor
Universita' di Pisa
Department of Experimental Pathology, M.B.I.E.
Via Roma 55
56126 Pisa Italy
Phone: +39(050)2218547
e-mail: fbruschi@med.unipi.it

90. Characterisation of clinical features, diagnosis and epidemiological aspects surrounding four *Trichinella* outbreaks in Slovakia over the last two decades

Reiterová, Katarína, Jana Kinčeková, V. Šnábel, P. Dubinský

Parasitological Institute SAS, Department of parasitozoonoses, Košice, Slovak Republic

This report presents the course and circumstances of four *Trichinella* outbreaks in Slovakia occurred due to consumption of pork from a backyard pig and admixture of infected wild boar's or dog's meat to meat products from 1990 to present. Patients suspected of acquiring infection were serologically examined by ELISA, sera of patients with symptoms of clinical trichinellosis were additionally tested by Western blot. In symptomatic patients haematological and biochemical parameters were estimated. Two outbreaks in 1990 and 1992 (71 people affected) and the last one in 2001 (23 people consumed the infected meat) were caused by the *T. spiralis* species. The incubation period ranged between 9-25 and 14-44 days dpi, respectively. The largest epidemic implied by the *T. britovi* species broke out in 1998 (336 people were affected). In this case the patients were serologically examined only 8-12 weeks after probably infection. In all events the most frequent clinical symptoms included ocular manifestations, muscular ache, dyspeptic symptoms, lipothymia, tachycardia, headache, oedema, and dermal manifestations. Patients showed anti-*Trichinella* IgA, IgM and IgG antibodies in their sera by an ELISA against somatic and excretory-secretory antigens. In majority of the patients IgM were first manifested, followed by IgG and/or IgA antibodies. Sera from symptomatic patients exhibited bands of 64, 47, 45 and 43 kDa of crude *T. spiralis* antigens by Western blot. In the two former outbreaks consumption of wild boar meat and severe clinical symptoms indicated *T. spiralis* as a causative agent of infection. In the later outbreaks, parasite larvae isolated from pork were identified by multiplex PCR analysis. The patients were treated with mebendazole or thiabendazole. The occurrence of human trichinellosis in the areas where feral animals have previously been considered *Trichinella*-free, was unexpected. Following the suspicion of trichinellosis, larvae were detected in the meat and meat products and the course of disease in patients was successfully controlled. To manage unexpected launch of outbreak, it is necessary to employ the complex approach with an adequate epidemiological anamnesis, early diagnosis, subsequent therapy, and identification on the infection source.

Work supported by the Slovak Grant Agency for the Science and Development Contract No. APVV-51-027 605 and partially supported by the Slovak Grant Agency VEGA, Grant No. 2 / 7186/27.

Katarína Reiterová, Dipl.Eng., PhD.
Parasitological Institute SAS
Department of parasitozoonoses
Hlinkova 3, 040 01 Košice
Slovak Republic
Phone: +421 55 63 344 55
e-mail: reiter@saske.sk

91. Heterogeneity of human trichinellosis and animal *Trichinella* infection in Romania: a geographical paradox due to ancestral food habits and political changes

Blaga, R.[4,5], B. Durand[1], A. Stoichici[2], Silvia Antoniu[3], C. Gherman[4], Nicolae Stefan[2], V. Cozma[4], P. Boireau[5]

[1]Epidemiology Unit, AFSSA, 23, MAISONS-ALFORT cedex, France
[2]Institute for Diagnosis et Animal Health, Bucharest, Romania
[3]University of Agronomical Sciences et Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Bucharest, Romania
[4]University of Agricultural Sciences et Veterinary Medicine, Faculty of Veterinary Medicine, Parasitology Unit, Cluj-Napoca, Romania
[5]JRU BIPAR INRA AFSSA ENVA UPVM, 23, avenue du Général de Gaulle BP 67– 94703 MAISONS-ALFORT cedex, France

With an incidence of 55,1 cases/106 man-year of trichinellosis over a period of 8 years et a prevalence of 8 cases/104 tested animals for pigs, 9 cases/103 for wild boars, 13,1 cases/102 for bears, Romania represents the country with the most extensive *Trichinella* infection in the world. The uneven repartition of populations of German origin, who colonized specific areas of Romania (Transylvania) beginning with the end of the 12th century, and their particular food habits (raw pork meat et sausage consumption) was taken into account though, when analysing trichinellosis epidemiological data in Romania. Analysis of human incidence and of animal prevalence data result in an apparent geographical paradox: whereas the trichinellosis incidence rate is twice higher in Transylvania than in the other counties, the *Trichinella* infection prevalence in pig is much lower. Hypotheses and facts contributing to the heterogeneity of human trichinellosis and *Trichinella* infection cases in Romania are discussed.

Work founded in part by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156) and in part by the EU project MedVetNet (Workpackages TRICHINET and TRICHIMED).

Radu Blaga, DVM, PhD
University of Agricultural Sciences and Veterinary Medicine, Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, Maisons-Alfort, France
Department of Parasitology Unit, Faculty of Veterinary Medicine
3-5 CALEA MANASTUR
400372 CLUJ-NAPOCA ROMANIA
Phone: 0040264596384
e-mail: blecgec@yahoo.com

92. Epidemiological and diagnostic features in a 16-year trichinellosis surveillance in Timis County, Romania

Neghina, R.[1], Roxana Moldovan[2], I. Marincu[3], G. Darabus[4], Adriana-Maria Neghina[5], L. Tirnea[1], R. Olariu[1], I. Iacobiciu[1]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Parasitology
[2]Department of Microbiology
[3]Department of Infectious Diseases
[4]Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Department of Parasitology
[5]Department of Biochemistry

Introduction The main source of trichinellosis in Romania is the insufficiently cooked pork infected especially with *Trichinella spiralis*. Timis is the most extensive Romanian county, situated in the western part of the country. The pork and the traditional food prepared from pork are extensively consumed here.
The aim of this study is to bring new data on human trichinellosis in Timis County and to compare them with other worldwide results.
Matherial and methods The medical recordings of 521 patients were investigated. They were hospitalized at "Victor Babes" Hospital of Infectious Diseases from Timisoara during the period 1990-2005.
Results The highest number of cases were registered in 1994 (16,90%) and most of the patients were between 20-29 years old (23,22%). Males and females were affected in an almost equal percentage. Among simptoms frequently observed were: myalgia (72,55%) and edemata (54,12%). Laboratory investigations included eosinophil and leucocyte values and in some cases ELISA was performed. Hospitalization period ranged between 8 and 14 days for 52,02% of the patients.
Conclusions Cases of uman trichinellosis have decreased in Timis County during the last 16 years as a result of hygenic rules' improvement. Most of the patients were from urban areas, but often the source of infection was in rural areas. Costs for hospitalization of the affected patients have consumed considerable health care resources

Raul Neghina, Assistant Professor, MD
"Victor Babes" University of Medicine and Pharmacy Timisoara
Department of Parasitology
2nd Eftimie Murgu Square
300041 Timisoara Romania
Phone: +407 23 46 45 17
e-mail: raul.neghina@gmail.com

93.

A major trichinellosis outbreak in northern Laos, June 2005

Barennes, H.[1], S. Sayasone[1,2], A. De Bruyne[4], S. Hongsakhone[1,3], P. Odermatt[1,2], P. Vonprhachanh[1,5], P.N. Newton[6,7] M. Strobel[1], J. Dupouy-Camet[4,7]

[1]Institut Francophone pour la Médecine Tropicale, Lao PDRb
[2]Dept. Epidemiology and Public Health, Swiss Tropical Institute, Basel, Switzerland
[3]Udomxay Regional Hospital, Udomxay, Lao PDR
[4]Service de Parasitologie-Mycologie and Centre National de Référence des Trichinella Hopital Cochin, Paris, France
[5]National Centre of Epidemiology and Laboratory, Vientiane, Lao PDR
[6]Wellcome Trust-Mahosot Hospital-Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Lao PDR.
[7]Center for Clinical Vaccinology and Tropical Medicine, Nuffield Department of Clinical Medicine, Churchill Hospital, University of Oxford, Oxford, United Kingdom.

Background: Trichinellosis, a food borne zoonotic disease, is reemerging in Southeast Asia. In 2003, after 30 years of no reports, a small outbreak was reported in Central Laos.
Settings: Villages and hospital in Udomxay Province, northern Lao PDR (Laos)
Methods: Rapid outbreak investigation: i) hospital-based, using a standardized questionnaire and definitions, peripheral blood cells counts,*Trichinella* ELISA and Western blot on filter paper blood spots, ii) village-based case control study with odds-ratio (OR) and attack rates (AR) iii) meal and pork sampling.
Results: The largest trichinosis outbreaks reported in Laos with ~ 600 estimated cases but no deaths. Two ceremonies (n=720) occurred at the onset of the epidemic (a wedding, AR: 75%, OR=15.0, 95% CI:10.5-2242, a funeral, AR: 84%). Other independent sources were parties, markets, mobile food sellers with AR ranging from 20 to 56%. People ate uncooked 'lap-mou' (minced pork and mint) and uncooked 'som-mou' (fermented pork meat). Free ranging pigs of several farms were contaminated and *Trichinella spiralis* larvae were identified from a pork sample. The main symptoms were myalgia (93.9%), edema (54.6%), fever (48.5%), diarrhea (25%). 77.3% had hypereosinophilia >1000/mm3 (range 255-6480 mm3). 80.4% of 133 blood spots were positive by *Trichinella* ELISA and Western Blots analysis of 16 sera confirmed *Trichinella* antibodies.
Discussion: Results suggest a high prevalence of trichinellosis in free ranging pigs in Udomxay Province. Reasons of low morbidity are discussed.
Conclusion: This large outbreak highlights the important public health impact of this disease and urgent need for veterinary control measures.

Hubert Barennes, MD, PhD, Epidemiologist,
IFMT BP 9519 Vientiane Lao P.D.R.
e-mail: hubert.barennes@auf.org
Phone (856.21).21.93.46 or + (856) 20 580 1742
Fax (856.21).21.93.47

94. Laboratory diagnosis of human trichinellosis by ELISA IgG, Western blot and ELISA IgG Avidity

Raynova Iskra, Marinela Ivanova, Rossitza Kurdova - Mintcheva

National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria

Diagnosis of human trichinellosis is confirmed by serological methods, among which the most popular one is ELISA for detection of specific IgG antibodies. The aim of the current study was to make a paralel comparison of three serological methods (ELISA IgG, Western blot and ELISA IgG avidity test) that can be used for the diagnosis of human trichinellosis. ELISA IgG test based on the indirect technique routinely used in the laboratory practice in Bulgaria was applied. *Trichinella* Western blot assay and ELISA IgG avidity test were developed using the ES antigen from *T. spiralis* larvae cultivated in vitro. *Trichinella spiralis* strain is maintained on guinea pigs at the Department of Parasitology and Tropical Medicine at NCIPD. For the Western blot assay nitrocellulose membrane (0.45 mm), human anti-IgG antibody conjugated with peroxidase and diaminobensidine as a substrate were used. *Trichinella* IgG avidity test was elaborated on the basis of the existing ELISA IgG test and for disruption of the antigen-antibody bonds 6 M urea was used. The sera panel was collected from patients involved in the trichinellosis outbreaks registered in the country. Results showed that our Western blot with ES antigen can be used as a confrmatory test and for the first time in a comparative study ELISA IgG avidity test was applied.

Iskra Rainova, MD, PhD
National Center of Infectious and Parasitic Diseases
Parasitology Department
1514 Sofia, Bulgaria
Phone: + 359 2 / 843 80 02
e-mail: iraynova@yahoo.com

95.

Cardiac involvement in a family focus of trichinellosis

Dumitru, Irina Magdalena, Sorin Rugina, Claudia Nina Rugina

Background. EKG abnormalities appear to be a common feature of trichinellosis especially during the invasive phase of the disease.
Objective. To study cardiac involvement in patients hospitalized with trichinellosis in Clinical Hospital of Infectious Diseases in 2006.
Method. We present 8 adults patients admitted with trichinellosis in December 2006 in our hospital, diagnosed by clinical and epidemiological conditions and by the presence of anti-*Trichinella spiralis* antibodies (ELISA). Patients were from the same family focus and eated untested pork meat with the occasion of winter holidays.
Results. All the patients, especially in the invasive phase of infection, were found to have abnormalities when examined by 12-lead, resting electrocardiography. The ECG disorder most frequently observed was sinus tachycardia, followed by bundle-branch conduction disturbances, supraventricular extrasystoles and atrial fibrillation. Six patients were identified as cases of myocarditis (5 in the invasive phase and one in the convalescent). Leucocitosis and high values of eosinophils was present (between 12%-57%). Under Albendazol and pathogenic treatment the evolution was favorable in all cases, prolonged in severe forms. We observed a poor evolution under Mebendazol treatment.
Conclusions. In all registered cases, cardiac manifestations were present. The highest values of eosinofils were founded in severe forms, probably because the high infestations. The evolution was favorable under Albendazol treatment.

Irina Magdalena Dumitru, Ass. Prof., MD, PhD
Clinical Hospital of Infectious Diseases,
"Ovidius" University
Department of Infectious Disease I
Ferdinand, 100
900709 Romania
Phone: 0040744761297
e-mail: dumitrui@hotmail.com

96. The effect of the *Trichinella* sp. larvae concerning the cardiovasculary system in men

Cristea Ana-Debora[1], Eugenia Cristea[2], D. Marculescu[3], V. Jula[3]

[1]Cardiology Resident Doctor Timisoara, Romania
[2]Individual Medical Cabinet – Vulcan, Romania
[3]The Medico-Legal Service Hunedoara, Romania

A number of 485 infested with *Trichinella* sp. persons were studied, being classified, depending on the clinical evolution (symptoms' severity), in three forms: mild – 88 patients (18,2%), medium – 384 ill persons (79,1%), severe – 13 patients (2,7%). In these persons diagnosed with trichinosis, the incubation period was between 8 and 21 days and the average of the hospitalization days was about 7- 21 days, depending on the symptoms' severity.

The trichinosis symptomatology makes its debut, varying with the biological cycle and the infested stage, with abdominal pains, vomitintg, diarrhoea (in parasites' copulation and the penetration in the intestinal mucous membrane of the female); then facial, limbs' oedema, fever can appear (in the circulatory larvas' stage) and in the penetration in the muscles fibres of the females, high blood pressure, low blood pressure, severe cardiovasculary disorders are met, , putting an end with the neuropsychical disturbances.

The cardiovasculary disordes described in the human trichinosis are ominous, being found in a percentage of 8,8 % patients. These disorders begin with tachycardia, cardiac sounds (in mild form), going on with myocarditis, bradycardia, arterial hypertension stages I-II, vasculary thrombosis in the moderate evolution, and in the severe forms myocarditis increases, suddenly appear dyspnoea, palpitations, anghina, ischemical cardiopathy, high blood pressure stages I-II, going to an end with the neuropsychical disturbances (cephalalgia, vertigo, delirium, coma, reflexes abolishment).

From the histo-pathological point of view, because of the parasite and/or excretion/ secretion toxins, lympho- histiocytary arteritis and periarteritis appear at the blood vessels level, and at the heart level were found myocarditis and interfascicles oedema, and in the acute phase were described as present myocarditis and eosinophilical lymphadenitis.

97. Main epidemiological features of human trichinellosis in Bulgaria in recent years (2002 – 2006)

Ivanova Marinela, Rossitza Kurdova – Mintcheva

National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria

Marinela Ivanova, Research fellow, MD
National Center of infectious and Parasitic Diseases
Parasitology Department
1514 Sofia, Bulgaria
Phone: + 359 2 / 843 80 02
e-mail: metichka2002@yahoo.com

In Bulgaria outbreaks and sporadic cases of human trichinellosis are registered every year. The aim of the current study was to analyze the basic epidemiological characteristics of the disease and evaluate the dynamics of the situation. Trichinellosis is a notifiable disease in the Republic of Bulgaria and during the last five years (2002 – 2006) altogether 40 outbreaks and 45 sporadic cases were officially registered in the country. The established number of outbreaks caused by consumption of infected pork meat products was 14, of wild boar meat – 15, and in 11 cases the source was not clearly identified. During the epidemiological investigation and clinical observation of all consumers it was detected that 767 individuals (56.11%) developed trichinellosis. Outbreaks were registered on the territory of 17 administrative regions, four of them being most affected. The greatest proportion of the outbreaks was reported during the winter season. Some other epidemilogical peculiarities such as sex ratio, urban and rural population proportion, age, etc. were analyzed.

98.

Trichinellosis in Georgia

Zirakishvili, L.[1], N. Iashvili[1], Lela Arabuli[2], Irina Kalandadze[3]

[1]S.Virsaladze Research Institute of Medical Parasitology and TropicalMedicine
[2]Institute of Zoology, Tbilisi, Georgia
[3]National Centre for Disease Control, Tbilisi, Georgia

Trichinellosis is a widespread parasitic zooanthroponosis in Georgia. *Trichinella* is detected in wild animals: jackal, fox, badger, wolf, brown bear, weasel. The natural cycle of trichinellosis has largely been maintained by wild and domestic pigs, cats, rodents, house rats, forest mousses and etc. Epidemiological connection of domestic area of trichinellosis with natural ones is confirmed. Essential place in biological circulation of trichinellas is occupied by rats and cats that are in sinanthropic biogenesis - in private houses, pig-breeding farms and dumps.

The first case of Human Trichinellosis in Georgia was described by the academician G.Maruashvili in 1952. Single cases had been manifested until 1984, but since then the disease prevalence has increased sharply. Between 1980-1984 only 24 cases were reported. However, for the periods 1985-1989 there were 475 cases (incident 0,096, per 100 000 population), for the periods 1990-1994 - 1151 cases (incident 1,89), for the periods 1995-1999 - 456 cases (incident 4,88) and for 2000-2004 - 859 cases (incident 1,25). In 2005-2006 had been 291 cases of trichinellosis registered. The disease is more prevalent in East Georgia. Over 80% of these cases came from Kakheti and Mtskehta- Mtianeti regions. Most cases are originated from domestic pork, although outbreaks from wild game are thought to be important. There is the direct correlation between increased number of cases in pigs trichinellosis and disease prevalence in population is fixed.

According to the scientific data *Trichinella spiralis* cases are more prevalent. Proceeding from the present epidemiological situation of trichinellosis Georgia should be considered as a stationary unfavourable synantropic zone.

Nino Iashvili
S.Virsaladze Research Institute of Medical Parasitology and Tropical Medicine
Departament of Medical Parasitology
Agmashenebli ave. 139
0102 Tbilisi Georgia
Phone: 095 32 959343
e-mail: nino.iashvili@yahoo.com

99.

Human trichinellosis in Constanta county

Sorin Rugina, Irina Magdalena Dumitru, Claudia Nina Rugina

Background. Trichinellosis is a common infection worldwide, but it is seldom seen in the United States and Western Europe because of strict rules regarding the feeding of domestic animals and meat-processing inpections. In Romania, before 2001, trichinellosis was endemic diseases, and was represented a major healt problem.

Objective. To study clinical and epidemiological aspects in patients with trichinellosis in Constanta county.

Method. We performed a retrospective study on patients admitted with trichinellosis in Clinical Hospital of Infectious Diseases during 1992 - 2007.

Results. The highest incidence was registered in 1993 (216 cases) and 1999 (165 cases), while after 2001 was registered a low number of cases (3, 4, 5, and 11 cases in 2003, 2004, 2005 and 2006). A higher frequency was registered in the rural environment (72%) while in the urban environment were registered only 28%. In 1999, most of the cases admitted in summer, while in December was registered only 1 case (hasn't respect anymore the winter seasonally associated to the pig sacrifice for Christmas holiday). Clinical manifestations of the diseases include muscle pain and tenderness, weakness, fever, headache, and swelling of the face, particularly around the eyes. A myocarditis was present in 8% of the cases.

Conclusions. Evolution of trichinellosis in human is strong influenced by the changes in the society structure and especially of the economical and social slump characteristic for this period. In the last year the incidence of trichinellosis was low, mainly because of the rigorous meat inspection procedures.

Sorin Rugina, Professor, MD, PhD
Clinical Hospital of Infectious Diseases, Ovidius University
Department of Infectious Diseases II
Ferdinand 100
900709 Romania
Phone: 0040723575680
e-mail: sorinrugina@yahoo.com

100. Human Trichinellosis in Arad County, Romania. A clinical 10-year retrospective study

Neghina, R.[1], Roxana Moldovan[2], I. Marincu[3], G. Darabus[4], Adriana-Maria Neghina[5], L. Tirnea[1], R. Olariu[1], I. Iacobiciu[1]

[1]"Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Parasitology
[2]"Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Microbiology
[3]"Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Infectious Diseases
[4]Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Department of Parasitology
[5]"Victor Babes" University of Medicine and Pharmacy Timisoara, Department of Biochemistry

Introduction The aim of this study is to present some clinical, laboratory, diagnostic and therapeutic data as regards trichinellosis in Arad county, which is the fifth larger among the Romanian counties, situated at the border with Hungary.

Matherial and methods The study group includes 335 patients,inhabitants of Arad County who were hospitalized at Arad County Clinical Hospital, Department of Infectious Diseases, during the period 1996-2005. Their hospitalization documents were investigated in order to perform this retrospective study.

Results The most frecquent simptoms were: fever and shivers (85,37%), local and general myalgia (82,98%), edemata (64,77%), digestive disorders (37,91%), headache (35,22%) and weakness (24,77%). The milder form of the disease was diagnosed in 56,12% of the patients. Eosinophils ranged between 10 and 20% in 17,01% of cases, leucoytes were over 10 000/mm3 in 58,21% of cases. Thiabendazole was administered in 49,45% of cases. The hospitalization period ranged between 8 and 14 days for 45,97% of patients.

Conclusions In Arad county trichinellosis has increased during the years 2004-2005, unlike in the neighbour western county,Timis. A major problem regarding the precise diagnosis of the disease resides in the routine and low cost methods that the physicians must use: eosinophil values and the pork trichinoscopy.

Raul Neghina, Assistant Professor, MD
"Victor Babes" University of Medicine and Pharmacy Timisoara
Department of Parasitology
2nd Eftimie Murgu Square
300041 Timisoara, Romania
Phone: +407 23 46 45 17
e-mail: raul.neghina@gmail.com

101.

Clinico-evolutive aspects of the trichinellosis in a rural focus

Marincu, I. [1], R. Neghina[2], L. Tirnea[2], I. Iacobiciu[2], L. Marincu[1], R. Olariu[1]

[1]Clinic of Infectious Diseases, U.M.F. Timisoara, Romania
[2]Departement of Parasitology, U.M.F. Timisoara, Romania

Introduction: the trichinellosis continues to remain a prime zoonosis wide-spread in the human communities that consume pork.
Objectives: the study of clinico-evolutive aspects of the trichinellosis at a group of patients out of a rural gipsy community.
Patients and methods: the authors have studied a group of 24 patients with trichinellosis hospitalized in the Clinic of Infectious Diseases Timisoara. All the patients were out of a rural gipsy community. The positive diagnosis was based on the epidemiological (infectious focus post consummation of pork infested with trichinella), clinical (fever, repeated chills, nausea, diarrhea, facial or periorbital edema, myalgia, headache, etc) and biological elements (ESR, fibrinogen, leucocytosis, eozinophylia, etc). The statistical processing of data was done using the EpiInfo 5 program.
Results: the clinico-biological modifications were registered in the individual patients file, following the established protocol; thus, 62,50% of the patients presented dispectic digestive syndrome, 80,50% presented fever over 38°C, 50% with periorbital edema, 15% had non-specific eruptions, 8,33% with myocarditis, 20,23% had pulmonary affections; 11,73% evolved in severe clinical forms, 56,87% medium forms, 23,32% mild forms and 7,84% asymptomatic forms. Under allbendazole treatment (Zentel, 1 tb=200 mg), 400 mg/day, for 7-10 days, all patients had a favourable clinical evolution.
Conclusions: the study of clinico-evolutive particularities of trichinellosis permits the delimitation of clinico-evolutive forms, with the aim of applying an efficient specific treatment.

102. Evaluation of the dynamics of haematological parameters in trichinellosis

Nemet, Codruta, Mihaela Elena Idomir, Maria Elena Cocuz, Liliana Rogozea

Trichinellosis is a cosmopolite parasitosis with higher values of the prevalence in temperate and cold zones. In Romania trichinosis is present on the entier teritory, having an endemic-epidemic evolution. In the county of Brasov, an important mountain area of Romania, the incidence of trichinellosis excedes constantly the registered values at national level.

The aim of our study has consisted in the evaluation in dynamics of the hematological changes found in these patients. The study group has included 365 cases of trichinellosis hospitalized in the Infectious Disease Hospital of Brasov during a 10 year timeframe (1995-2006).

Almost all cases have presented peripheral eosinophilia (91,9 %). Moderate increases of this parameter (1500-5000 eosinophils/ mm3 of blood) were the most frequent found (52,8 % of the patients who presented eosinophilia). Asymptomatic, mild and medium forms of trichinellosis were associated particularly with low and moderate eosinophilia. High levels of eosinophilia were particularly present in medium-severe and severe forms of the disease. The maximum value of the number of peripheral eosinophils has been 12033 eosinophils/mm3 of peripheral blood. Other haematological disorders associated with trichinosis have consisted in leucocitosis (68,9 %), anemia (49,4 %) and the increase of erythrocyte sedimentation rate (6,6 %). We have not observed significant variations of the frequency and intensity of these parameters corelated to the age and gender of the patients.

Codruta Nemet, Prof. PhD
Transilvania University of Brasov
Department of Parasitology
Romania
Phone: +40 268 416543
e-mail: codruta_nemet@yahoo.com

103. Clinical features in the human trichinosis evolution in the Jiu Valley

Cristea Eugenia[1], Debora Cristea[2], D. Marculescu[3], V. Jula[3]

[1]Individual Medical Cabinet– Vulcan, Romania
[2]Cardiology Resident Doctor Timisoara, Romania
[3]The Medico-Legal Service Hunedoara, Romania

Trichinosis, a parazitozoonozis known for more than 170 years, continues to produce also in the third milenium in Romania, especially in the Jiu Valley, serious effects manifested in suffering, ruin, diminution of the ability to work, and not in the last row, economical extremely damages. In the 1987-2006 period, in the Jiu Valley, a number of 2524 persons were taken ill. After a incubation period of 8-21 days, depending on the ingested larvae number and the body reactivity, digestive disorders appear, being represented by abdominal pains, nausea, vomiting, diarrhoea, lack of appetite, fever, muscle pains, oedema, skin eruptions, and in medium or severe forms cardiovasculary, neuropsychical, respiratory and renal complications appear. Clinical and paraclinical descriptive study was effected on a number of 485 patients classified depending on symptoms' severity in the following forms: mild (18,2%), medium (79,1%), severe (2,7%). In all patients joined in the three forms (mild, medium, severe), muscles affections predominated (91,2%), being represented by muscle pains in superior limbs, vague moderate muscular pains in inferior limbs, cervical, thorax, abdominal muscular pains.

Cardiovasculary disorders appeared in a number of 43 patients, being represented by tachycardia, myocarditis, arterial hypertension stages I-II, vascular thrombosis, palpitations, ischemical cardiopathy, heart failure.

In mild and severe forms diagnosed in 397 patients, the neuropsychical complications represented by headache, nervousness, psychosis, depression, paresis, encephalitis.

Respiratory disorders such as cought, dyspnoea, bronchitis, bronchopneumonia, pleuresy, appeared in a number of 139 patients (35%).

Renal complications represented by oliguria, polakiuria and proteinury were found in 12 patients, representing a percentage of 3%.

104. Validation and application of an ELISA for the diagnosis of trichinellosis in humans

Ludovisi, Alessandra, M. Amati, Maria Angeles Gomez Morales, E. Pozio

Today, the serology is the most used method to confirm a clinical diagnosis of trichinellosis; however, in spite of the spread of commercial kits and the development of home made tests in several European laboratories, no standard has been established, as well as never a serological test has been validated using a significative panel of reference sera from healthy people, people with other diseases and people with confirmed trichinellosis. The Community Reference laboratory for parasites (CRLP) is validating an ELISA for the diagnosis of trichinellosis. Using an excretory/secretory antigen from *Trichinella spiralis* produced at the CRLP, 1,500 sera from *Trichinella*-free healthy donors, 1,500 sera from *T. spiralis* or *T. britovi* infected persons, and 1,800 sera from people affected by 25 protozoan and helminthic infections, and 5 non-infectious autoimmune diseases, were screened using the standard protocol. The sensitivity, specificity, reproducibility, robustness and positive and negative predictive values are under evaluation. To determine the optimal cut point, Receiver operating curves (ROC) will be used.

Dr. Alessandra Ludovisi
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299, 00161Rome
Italy
Phone: 00 39 06 4990 2310
e-mail: alessandra.ludovisi@iss.it
Web: www.iss.it/crlp/

105. Results of the follow-up of the former trichinellosis patients from Brasov county – Romania

Nemet Codruta[1], Liliana Rogozea[1], Răzvan Dejica-Cocan[2]

[1]RANSILVANIA University Braşov, Faculty of Medicine
[2]Zentiva SA – Romania

The objective of the study was the monitoring for a period of two years (at 1,3,6,9,12,24 months) of the former trichinellosis patients (699) registered in Braşov county before the start of the initiation of the National Supervision and Control Programme for trichinellosis in humans.

During the study immediate complications up to 6 months from release and related complications that patients indicate even after 24 months were identified. Immediate complications were: cardiovascular (31%), muscular (19%), neurological (18%), ophthalmic (17%), allergic (9%), respiratory (1%), other types (5%). Complications are more frequent in the case of children from the studied lot, even if the disease form they suffered from was usually mild or medium, in the case of women rather then male patients, in the urban area rather than the rural environment. Paraclinical data: eosinophilia: 8-22% for all patients, GPT and GOT within normal limits, hypoproteinemia in the case of 178 former patients (31%). Hypocalcemia and hypomagnesemia persist in the case of 401 former patients (71%.). After 9 months since falling ill, 212 (37,8%) of the former patients indicate fatigue, myalgia especially in the inferior limbs, cardiovascular disorders, neurological, psychiatric and allergic illnesses. At one year since the start of the disease, 102 (18%) of the former trichinosis patients, "improved", indicate: asthenia, muscular pains, allergic reactions, hypertension, cardiac arrhythmia, angina pectoris crises. After two months since falling ill, 53 (9,4%) of the former patients remain ailing, 26 of them being retired on medical grounds at present. Trichinosis in humans cannot be considered only an acute disease that, once properly treated etiologically, pathogenically and symptomatically improves, but it can also become chronic, transforming the former patient into an invalid.

Codruta Nemet, Prof. PhD
Transilvania University of Brasov
Department of Parasitology
Romania
Phone: +40 268 416543
e-mail: codruta_nemet@yahoo.com

106. Actual status of Trichinellosis in Romania

Cretu, Carmen-Michaela[1], Suzana-Elena Cilievici[1], Loredana Popa[1], I. Dida[2], Radu Blaga[3], P. Boireau[3]

[1]„Carol Davila" University of Medicine and Pharmacy, Colentina Teaching Hospital – Parasitology Clinic, Eco-Para-Diagnostic SRL, Bucharest, Romania
[2]Faculty of Veterinary Medicine – Parasitology Department, Bucharest, Romania
[3]Joint Research Unit BIPAR INRA AFSSA ENVA UPVM, 23, Maisons-Alfort, France

Introduction: Trichinellosis, zoonosis transmitted by eating row or improperly cooked meat containing *Trichinella* spp. larvae is still spread in Romania. In spite of prophylactic and control measures, trichinellosis cases are still common in humans and animals. Material and Methods: A retrospective analyse on human and animal cases of trichinellosis have been done during the last decades, showing the same descendent trend. In humans recent data shows an important decrease of the disease (3.649 cases in 1993, 350 in 2006). The evaluation of the most important clinical and epidemiological features of the disease indicates few and small foci, mostly in countryside and many isolated cases, every year in different parts of Romania. At the same time, many mild or sub clinical cases are late identified, making the diagnosis difficult and conducting to complications or chronic trichinellosis and sequels. Animal trichinellosis reveals the same descendent trend (10.540 cases in 1993, 674 in 2006). The infection is steel common in bears and wild boars. The analysis of the most important risk factors, synthesize the epidemiological peculiarities and the possibility of transition of the infection to other animals. The confirmation of human trichinellosis is done by serology – ELISA, WB, muscle biopsy. Animal cases are investigated mostly in abattoirs and morphological procedures as artificial digestion (recently introduced, according to EU requirement) are performed. Conclusions: Trichinellosis is still common in human and animals, the correct control/ surveillance depending on the appliance and sustainability of the appropriate measures.

Founded by the EU project "TRICHIPORSE" (contract QLK1-CT-2001-01156)

PhD Carmen-Michaela Cretu, Associat Professor
"Carol Davila" University of Medicine and Pharmacy
Department of Parasitology
Glinka Str 10, Flat 2, 020216Bucharest
Romania
Phone: +40 722 503 207
e-mail: carmenmihaelacretu@yahoo.com
Web: www.ecoparadiagnostic.ro

107.

The evolution of trichinelosis during 2000-2006 in Romania

Dascalu Lenuta

The purpose of the analysis of statistic data was to establish the incidence and yearly dinamics of trichinelosis at pigs, wild boars, bears and humans in Romania during the years 2000-2006. The incidence during this period was:
-at pigs: 2000 – 0,08%, 2001 – 0,06%, 2002 – 0,03%, 2003 – 0,04%, 2004 – 0,09%, 2005 – 0,04%, 2006 – 0,02%;
-at wild boars: 2000 – 0,71%, 2001 – 2,34%, 2002 – 0,61%, 2003 – 3,29%, 2004 – 1,11%, 2005 – 1%, 2006 – 0,58%;
-at bears: 2000 – 12,3%, 2001 – 6,51%, 2003 – 25%, 2004 – 7,73%, 2005 – 23,81%, 2006 – 8,99%;
-the number of confirmed cases at humans: 2000 – 624, 2001 – 642, 2002 – 281, 2003 – 102, 2004 – 179, 2005 – 103, 2006 – 147.
Trichinelosis is stil one of the major zoonosis with high risc for the public health, in case that the meet comes from receptive animals and without a veterinary control.

Lenuta Dascalu
Institute for Diagnosis and Animal Health
Department of Parasitology Laboratory
Dr. N. Staicovici street 63, sector 5
0050557 Bucharest, Romania
Phone: +402 14 12 99 30
e-mail: dascalu.lenuta@idah.ro

108.

TRICHINELLOSIS - Pitfalls in diagnosis and management

Carmen-Michaela, Cretu[1], Denisa Predeteanu[2], Suzana-Elena Cilievici[1], Andaluzia Oancea[3]

[1]"Carol Davila" University of Medicine and Pharmacy, Colentina Teaching Hospital – Parasitology Clinic, Eco-Para-Diagnostic SRL, Bucharest, Romania
[2]"Carol Davila" University of Medicine and Pharmacy,"Sfanta Maria" Clinic Hospital, Bucharest – Internal Medicine and Reumatology Clinic, Bucharest, Romania
[3]"Coltea" Clinic Hospital, Otorhynolaryngology Clinc, Bucharest, Romania

Introduction: In spite of the endemic trichinellosis in Romania and the notification of the disease, sometime late/ misdiagnosed cases can occur, when even the life of the patient can be in danger.
Material and Method: Three cases of trichinellosis admitted in the only Clinic for Parasitic Diseases in Romania (where treatment with Albendazole for human trichinellosis was initiated) are presented.
Case 1: A 52 yo, female patient coming from urban area, was admitted in Parasitology Department, from Reumathology Department. Initially diagnosed with Poliradiculoneuritis, Nephrotic syndrome, Systemic lupus, she came with 3 months history of progressive fever, malaise, progressive oedemas, paralysis, troubles of consciousness. IgG ELISA was negative, but WB confirmed the diagnosis.
Case 2: A 48 yo, male patient, is coming from the Otorhynolaringology Clinic, following the laryngeal carcinoma surgery, when some micro, white lesions, were noticed in the muscles. IgG ELISA for Trichinella was negative and later on, the muscle biopsy confirmed the diagnosis.
Case 3: A 32 yo male patient, from rural area, referred to Parasitology Clinic from Neurology, was confirmed with trichinellosis after one month history of abdominal pain, diarrhoea, acute appendicitis (operated) and diplopia. IgG ELISA was positive and full WBC showed hypereosinophilia.
Conclusion: In endemic areas, the diagnosis of trichinellosis should be considered in certain situation, even in the absence of hypereosinophilia, or absence of positive serology when patients receive corticosteroids. Western Blot confirmation test, recently performed in Romania, is useful for the accuracy of diagnosis.

Carmen-Michacaela Cretu, Assoc Professor
\"Carol Davila\" University of Medicine and Pharmacy
Department of Parasitology
Glinka Str 10
Post Code: 020216 Bucharest Romania
Phone: +40 722 503 207
e-mail: carmenmihaelacretu@yahoo.com
Web: www.ecoparadiagnostic.ro

109. Efficacy of a novel 2-(trifluoromethyl)-1H-bencimidazole derivative against *Trichinella spiralis* muscle larvae in experimental trichinellosis

Yépez-Mulia, Lilián[1], Gabriela Andrés-Antonio[1], O. Bustamante-García[2], G. Navarrete-Vázquez[3], Alicia Hernández-Campos[2], R. Castillo[2], F. H. Luis[2]

[1]Instituto Mexicano del Seguro Social, Hospital de Pediatría, Department of UIMEIP
[2]Facultad de Química, UNAM, México
[3]Facultad de Farmacia, UNAM, México

Albendazole (ABZ) is widely used in the treatment of *Trichinella* infections. However, high doses and long treatments are required to be effective due to its erratic bioavailability, related to their low solubility and extensive first past metabolism. In an attempt to have other anthelmintics with better bioavailability characteristics, we synthesized and tested a novel2-(trifluoromethyl)-1H-benzimidazole derivative (1). This compound showed similar in vitro activity than ABZ against *T. spiralis* muscle larvae (ML). The aim of this study was to evaluate the in vivo efficacy of 1 against *T. spiralis* ML. Additionally, in order to determine if the efficacy of 1 could be improved, this compound was administrated in inclusion complex (2) or physical mixture (3) with hydroxypropyl-beta-cyclodextrin. For the in vivo evaluation, forty BALB/c male mice were orally infected with 500 ML. Twenty eight days after infection, groups of ten mice were treated with 1, 2 and 3 at 75mg/kg/day, for 7 consecutive days. The fourth group was included as control. Seven days after the last administration, ML were recovered from the muscles of infected mice, and parasite reduction (%) was calculated with respect to the non-treated group. The highest parasite reduction was observed when 2 was used (84%), in comparison to 46% and 44% obtained when 1 and 3 were used, respectively.

Supported by CONACyT grant V43629-M

Lilián Yépez-Mulia, PhD
Instituto Mexicano del Seguro Social, Hospital de Pediatría
Department of UIMEIP
Av. Cuauhtémoc 330, Doctores,
CP 06720 Mexico City, Mexico
Phone: 52 55 56 27 69 40
e-mail: lilianyepez@yahoo.com

110. *Trichinella spiralis* oocyte maturation is inhibited by cyclosporin A in rats infected with this parasite

Hernandez-Bello, R.[1], Rosa M. Bermudez-Cruz[1], R. Fonseca-Linan[1], Patricia B. Garcia-Reyna[2], P. Boireau[3], Guadalupe Ortega-Pierres[1]

[1]Departments of Genetics and Molecular Biology, CINVESTAV-IPN. CP. Mexico
[2]FES-Cuautitlan. UNAM-Mexico
[3]UMR BIPAR, 23 avenue du General de Gaulle, Maisons-Alfort, France

Cyclosporin A (CsA), a potent immunosuppressive drug, has also been used as antiparasitic drug against a variety of helminths. In a previous study on the effect of CsA in mice treated with this drug during *T. spiralis* infection a total absence of new-born larva (NBL) deposition by female worms obtained from these animals was observed. This correlated with the lack of larval development in uterus. Based on this and on recent observations by our group on the role of caveolin-1 of *T. spiralis* (CavTs) in oocyte maturation in female worms, we studied the effect of CsA in the oogenesis of *T. spiralis* using CavTs protein as a marker of oocyte maturation. This was approached using light microscopy, immunohistochemical techniques with anti- CavTs antibodies together with confocal laser microscopy. Observation in light microscopy of parasites obtained at 1 day pi from untreated and CsA treated rats did not shown differences in oocytes maturation. However parasites obtained at 2 and 3 days pi from untreated rats showed mature and fertilized oocytes respectively. In contrast parasites from CsA treated rats showed only immature oocytes. Female parasites obtained at 5 days pi from untreated rats contained fully developed NBL in uterus, while females from CsA treated rats contained only immature oocytes. Confocal analysis of cross sections of female worms from untreated rats using anti-CavTs antibodies showed that CavTs is accumulated in the oocyte membrane until fertilization occurs. Similar analysis of female worms from CsA treated rats obtained at 1 to 5 days pi showed a lower CavTs expression in the immature oocytes with an irregular membrane localization. These results suggest that CsA may prevent the correct membrane localization and function of CavTs in oocyte maturation probably by depleting cholesterol from the oocyte membrane as has been shown in other cell systems.

Supported in part by ECOS-ANUIES Grant M06-S03 and CONACyT Grant G38523.

Romel Hernandez-Bello, MSc
Centro de Investigacion y de Estudios Avanzados del IPN
Department of Genetic and Molecular Biology
Av. Instituto Politecnico Nacional No. 2508
07360 Mexico, D.F. Mexico
Phone: 55 5061 3800 ext 5326
e-mail: romel@cinvestav.mx

111. Enantioselective accumulation of ricobendazole (albendazole-sulphoxide) by muscle larvae of *Trichinella spiralis*

Bolas-Fernández F., J.J. Torrado-Durán, J.J. Garcia-Rodríguez

Complutense University, Faculty of Pharmacy, Department of Parasitology; Plaza de Ramón y Cajal s/n, Ciudad Universitaria; Madrid; Spain

In anthelmintic chemotherapy, apart form the pharmacokinetic behaviour of active compounds within the host, the uptake, biotransformation and accumulation of drugs by the parasites are important factors that determine the effectiveness of treatments against the diseases they originate. Here, using the mouse model of infection by *Trichinella*, an ex vivo pharmacockinetic study of the of albendazole active metabolite (albendazol-sulphoxide= ricobendazole) in muscle larvae of *Trichinella spiralis* was undertaken.

L1 larvae of *T. spiralis*, isolated by artificial digestion from Swiss CD-1 infected carcasses, were incubated in HBSS medium for different times (1, 2, 4, 6, 10 and 18 hours) in the presence of various concentrations (0.5, 1, 2 and 4 micrograms/ml) of ricobendazole formulated in hydroxypropyl-β-cyclodextrins. Treated an untreated larvae were homogenized, extracted in acetonitrile and deproteinized in methanol. Following centrifugation and filtration the supernatant extracts were analyzed by HPLC, first in reserve-phase chromatography using a C18 column, for determination of the sulphoxide and sulphone derivatives and second using a Chiral AGP column to measure ricobendazole enantiomers.

The results indicate that uptake or ricobendazole by L1 larvae occurs mainly by transcuticular passive diffusion and the kinetics during 18 hours shows a progressive enantioselective accumulation of ricobendazole(+) with little bio-transformation to the inactive albendazole-sulphone. These result are in accordance with the higher trichinellicide effect of the enantiomer (+) show in previous studies.

Work funded by MedVetNet/Trichimed/WP27

Francisco Bolás-Fernández, PhD
Complutense University
Faculty of Pharmacy
Department of Parasitology
Plaza de Ramón y Cajal s/n, Ciudad Universitaria
28040 Madrid, Spain
Phone: +34913941818
e-mail: francisb@farm.ucm.es

112. Detection of nematode larvae resembling *Trichinella* sp. larvae in meat samples

Marucci G., G. La Rosa, E. Pozio

Istituto Superiore di Sanita, Department of Infectious, Parasitic and Immunomedited Diseases, Rome, Italy

The artificial digestion is the gold standard diagnostic method to detect *Trichinella* sp. larvae in muscle tissues of animals. However, the detection of nematode larvae out of their natural niche (i.e. the muscle cell) can, sometimes, lead to the identification of nematode larvae belonging to other families or genera as *Trichinella* sp. larvae. Indeed, it is not unusual that larvae of nematodes living or migrating into the body of vertebrates, but in different niches such as the gut lumen, the liver, the lungs, the lymphatic or blood vessels, could be, by mistake, identified as larvae of the genus *Trichinella*. In most cases, the evaluation of their size, internal structure and shape can be enough to exclude a glaring blunder; however, sporadic cases exists in which also a good knowledge of the morphology of *Trichinella* sp. larvae cannot be enough to reach a correct identification. The International Trichinella Reference Centre receives *Trichinella* sp. larvae from different world regions for their identification, and false *Trichinella* sp. larvae have been detected. Samples from two bird of preys (a tawny owl, *Strix aluco*; an European scops owl, *Otus scops*) of Italy, and from a lion (*Panthera leo*) of South Africa were received, recently. In the two isolates from birds, an accurate morphological analysis of the parasites allowed to exclude an infection with *Trichinella* sp.; otherwise, the larvae from the lion were very similar in size and shape to those of the genus *Trichinella* sp. representing an interesting case for which the direct observation by a microscope did not allow to identify the parasite at the genus level. The screening of several family-specific primer pairs, will allow the identification by PCR of these larvae at least at the family level. This work is in progress.

Gianluca Marucci, PhD
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299, 00161Rome
Italy
+39 06 4990 2310
e-mail: gianluca.marucci@iss.it
Web: www.iss.it/crlp/

113. Evaluation of the digestion methods to detect *Trichinella spiralis* larvae in pork samples according to the EU directive 2075/2005 at the National Reference Laboratories for Parasites (NRLP) of EU countries

Marucci G., E. Pozio

Istituto Superiore di Sanita, Department of Infectious, Parasitic and Immunomedited Diseases, Rome, Italy

To control *Trichinella* sp. infection in the European Union, all slaughtered pigs should be tested by one of the approved digestion methods according to the EU directive 2075/2005. The aim of this work was to evaluate by a ring trial, the sensibility of the digestion method used at the NRLP, which, in their turn, are in charge to check the quality of the detection method in their own country. Of the 27 EU countries, only three (Hungary, Luxembourg and Malta) did not participated for different reasons. Every participating laboratory received ten samples of 100g of minced pork under vacuum, containing: 3-5 larvae (3 samples), 10-20 larvae (3 samples), 30-50 larvae (3 samples), and one negative control. In each positive sample, there were living *Trichinella spiralis* larvae with or without the collagen capsule, obtained by partial artificial digestion of infected mice. No false positive sample was found, whereas 9 laboratories (37.5%) failed to detect positive samples with a percentage of false negatives ranging from 10 to 100%. The statistical analysis of the results shows a direct correlation between the number of larvae in the sample and the error associated to the count, where lower the number of larvae, lower the error. The high difference between expected and reported count for the samples containing 30-50 larvae suggests a problem to count the larvae in the sediment. There is a direct correlation between the consistency of the results and the use of a validated/accredited digestion method, whereas no correlation was observed between the consistency of the results and the number of digestions performed yearly. This result strongly suggests the importance to validated/accredited the test.

Gianluca Marucci, PhD
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299
00161Rome, Italy
Phone: +39 06 4990 2310
e-mail: gianluca.marucci@iss.it
Web: www.iss.it/crlp/

114. Preparation and use of *Trichinella* proficiency samples as part of a quality assurance system to improve the sensitivity of *Trichinella* digestion testing in laboratories in France

Vallée, Isabelle [1], Pauline Macé[1], Lorry Forbes[2], B. Scandrett[2], B. Durand[3], A. Gajadhar[2], P. Boireau[1]

[1]UMR BIPAR, AFSSA-LERPAZ, Maisons-Alfort, France
[2]Centre for Food-borne and Animal Parasitology, Saskatoon Laboratory, Saskatchewan, Canada
[3]Epidemiology Unit, AFSSA-LERPAZ, Maisons-Alfort, France

Diagnosis of trichinellosis for food relies on an artificial digestion assay to free *Trichinella* spp muscle larvae (ML) from meat. As part of a quality control system, the French National Reference Laboratory (NRL) initiated ring trials to determine the sensitivity of the test performed in 72 routine diagnostic laboratories. A method was devised to obtain calibrated meat samples containing known numbers of *T. spiralis* capsules. This method used incomplete artificial digestion of infected mice muscle to collect intact *Trichinella* capsules, which were placed into meatballs to produce proficiency samples. Three categories of samples were prepared: small (3–5 capsules), medium (7–10) and large (12-15). The sensitivity was expressed as the percentage of ML recovered from each proficiency sample. Reproducibility was tested using ring trials between two NRLs (France, Canada) and a reference sensitivity of 84.9% was established. Ring trials were then organised in France with the 72 routine diagnostic laboratories each receiving 4 proficiency samples/session. After 5 sessions, an improvement of the digest test sensitivity was observed. Results at the 5th session indicated sensitivities of 78.6%+/-23.7, 81.2%+/-19.6 and 80.5%+/-14.7 ML for small, medium and large samples respectively. This study supports the use of proficiency samples to accurately evaluate the sensitivity of digestion tests for *Trichinella* spp detection in meat by routine diagnostic laboratories.

Supported by Trichimed

PhD. Isabelle Vallée
AFSSA-LERPAZ UMR BIPAR
23 avenue du Général de Gaulle
94706Maisons-Alfort
France
Phone: 33 1 49 77 28 16
e-mail: ivallee@vet-alfort.fr

115. Ring trial on the validation of three digestion methods for the detection of non-encapsulated *Trichinella pseudospiralis* larvae in pork

Noeckler, K.[1], Sabine Reckinger[1], I. Szabó[1], Charlotte Maddox-Hyttel[2], E. Pozio[3], J. van der Giessen[4], Isabelle Vallée[5], P. Boireau[5]

[1]Federal Institute for Risk Assessment, Berlin, Germany
[2]Dept. for Veterinary Diagnostics and Research, National Veterinary Institute, Technical University of Denmark, Copenhagen V, Denmark
[3]Department of Infectious, Parasitic et Immunomediated Diseases, Istituto Superiore di Sanitŕ, Rome, Italy
[4]National Institute of Public Health and the Environment (RIVM), Microbiological Laboratory for Health Protection, Bilthoven, Netherlands
[5]JRU BIPAR, AFSSA-LERPAZ, Maisons-Alfort, France

According to Regulation (EC) No 2075/2005, *Trichinella* meat inspection has to be performed by one of the approved methods of artificial digestion. Whereas the magnetic stirrer method is considered as the reference method, the stomacher and trichomatic 35Ž methods may be used as equivalent tests for the detection of *Trichinella* larvae in meat. The aim of this study was to conduct a ring trial with five laboratories to validate the three methods for the detection of non-encapsulated *T. pseudospiralis* larvae in pork.

Each laboratory had to test a panel of 20 *T. pseudospiralis*-positive meat samples with a lower and higher larval load by one of the approved method of artificial digestion. Pork containing non-encapsulated larvae was produced from a pig experimentally infected with 40,000 *T. pseudospiralis* larvae (strain ISS176). Subsequently, two batches with a nominal value of 7 and 17 larvae per g (lpg), respectively, were produced by mixing the minced meat from a negative and *Trichinella*-positive pig in a calculated relation. Prior to the examination, 1 g of each proficiency sample was mixed with 99 g of negative meat for the magnetic stirrer (labs A, B, E) and the stomacher method (lab B), whereas, 34 g were added for the trichomatic method (labs C, D). Results for the number of larvae were statistically analysed ($p<0.05$) in regard to the method and participating lab. The variation of the quantitative results (i.e. percentage of samples with larval findings that are outside the calculated tolerance range) was evaluated for each detection method by comparing the consensus result (mean number of larvae over all labs) with individual result of each lab. The tolerance range for larval finding was defined as mean number of larvae for all labs ą 2SD.

In regard to samples with a nominal value of 7 lpg, the number of larvae

examined by the stomacher method was significantly higher than in samples tested by the magnetic stirrer method, whereas no significant difference occurred with the trichomatic method. There was no significant difference for larval finding between all methods for samples with a nominal value of 17 lpg. In regard to results of labs irrespective of the used method, lab B detected a significantly higher number of larvae than lab E in samples with a nominal value of 7 lpg. Furthermore, lab E detected significantly less larvae than labs A, B and D in samples with a nominal value of 17 lpg. The lowest variation for enumerated larvae in samples with a nominal value of 7 lpg was obtained by magnetic stirrer and stomacher (each 20%) followed by trichomatic method (25%). For proficiency samples with a mean of 17 lpg, the lowest variation was achieved by magnetic stirrer (23%), followed by stomacher (30%) and trichomatic method (35%).

Differences in larval findings between methods are likely due to the lower detection rate observed in one lab. In regard to the variation for enumerated larvae, the best results were obtained by the magnetic stirrer method. Altogether, the results indicate that the magnetic stirrer, trichomatic and stomacher are all appropriate methods for the detection of non-encapsulated *T. pseudospiralis* larvae. Further investigations should focus on the optimisation of the detection system to improve the test sensitivity.

The study was supported by the 6th Research Framework Programme of the European Commission within the Network of Excellence "Med-Vet-Net" (WP TrichiMed).

Karsten Noeckler, PhD.
Federal Institute for Risk Assessment (BfR)
Department of Biological Safety
Diedersdorfer Weg 1
12277 Berlin Germany
Phone: +4930 84 12 20 53
e-mail: karsten.noeckler@bfr.bund.de
Web: www.bfr.bund.de

116. Improvisations or “creations” in meat examination on Trichinellosis using “ The Magnetic Stirrer Method for Pooled Sample Digestion”and reliability

Savić M., M. Đorđević, S. Pavlović

Institute of meat hygiene and technology,
Laboratory for parasitic disease, Beograd, Serbia

Marko Savic, DVM
Institute of meat hygiene and technology
Laboratory for parasitic disease
Kačanskog 13, 11000 Beograd
Serbia
Phone: +381112650722
e-mail: marko@inmesbgd.com

Based on the long-term preview of human *Trichinellae* cases and epidemics caused by meat inspected with “The Magnetic Stirrer Method for Pooled Sample Digestion” seven experiments were performed.We used five samples (50g. pork) and each contained 1,3,5,10,20 larvae. Twentyfive digestions were performed in each of seven experiments. There were no uncovered larvae and no mistakes in procedure in Exp.No.1 which was performed according to the Regulation 77/96/EEC.In the rest of six experiments percent of uncovered larvae was from 4% to 80% in relation to the number of larvae presented in sample of 50g i.e. sediment and following mistakes were made:procedure of suction of sediment (30ml of 40ml), using laboratory glass (100ml) instead measuring cylinders of approximately 50 ml capacity, or centrifuge tubes and using mouth/clip laboratory pipette (20ml) instead syringe.

117. Towards a risk-based *Trichinella* surveillance in Denmark

Boes, J.[1], Lis Alban[1], H. Kreiner[2], J.V. Petersen[1], P. Willeberg[2]

[1]Veterinary Research & Development, Danish Meat Association
[2]Danish Veterinary & Food Administration, Sřborg, Denmark

In its directive on *Trichinella* control, the EU has opened up for the possibility of countries being recognized as areas with negligible *Trichinella* prevalence in pigs for slaughter. In Denmark, *Trichinella* spp. have not been detected in domestic pigs for more than 70 years, and repeated surveys in the Danish fox population have shown a very low prevalence (< 0.1%) of *Trichinella*. Therefore, Denmark has applied to the EU to become recognized as an area with negligible *Trichinella* prevalence (< 1 per million). It is proposed that future *Trichinella* surveillance in Denmark will be risk-based, i.e. only testing of high-risk subpopulations such as all sows and boars and all outdoor reared pigs (approx. 600,000 animals). A model called Discounting Historical Evidence, which incorporates several years of surveillance data, was used to calculate the probability that the national pig herd is free from *Trichinella*. The model takes into account the annual probability that *Trichinella* is introduced to Denmark assuming an individual test sensitivity (ability to detect *Trichinella* if present) of 40%. The results showed that the estimated risk of not detecting *Trichinella* in domestic pigs in a risk-based surveillance system is negligible. The risk-based program will include annual monitoring of red foxes for *Trichinella* with special focus on the region along the German border and areas with previous findings of *Trichinella* in foxes. Contingency plans in case of *Trichinella* suspicion or detection in pigs or foxes have been developed. Quality assurance programs for all laboratories performing *Trichinella* testing have been implemented and evaluated.

Jaap Boes, PhD.
Danish Meat Association
Department of Veterinary Research & Development
Vinkelvej 11
DK-8620 Kjellerup Denmark
Phone: 0045-87714061
e-mail: jbo@danishmeat.dk

118. Validation of a unique digestion assay to consistently detect *Trichinella* larvae at critical levels in horse meat

Forbes Lorry[1], Dolores Hill[2], Sarah Parker[3], Stacy Tessaro[4], R. Gamble[5], A. Gajadhar[1]

[1]Canadian Food Inspection Agency, Centre for Food-borne and Animal Parasitology, Saskatoon Laboratory, 116 Veterinary Road, S7N2R3 Saskatoon, SK, CANADA
[2]USDA, Animal Parasitic Diseases Laboratory, BARC-East, Beltsville MD 20705 USA
[3]Western College of Veterinary Medicine, 52 Campus Dr, Saskatoon SK S7N5B4 CANADA
[4]CFIA, Lethbridge Laboratory, PO Box 640, Lethbridge AB T1J3Z4 CANADA
[5]National Academy of Science, 500 Fifth St NW, Washington DC 20001 USA

A tissue digestion assay using a double separatory funnel (DSF) procedure for the detection of *Trichinella* larvae in horse meat, and conducted within an ISO 17025 quality assurance system, was validated for application in food safety programs and trade. The assay consisted of a pepsin-HCl digestion step to release larvae from muscle tissue, followed by two sequential sedimentation steps in separatory funnels to recover and concentrate larvae for detection using a stereomicroscope. One-, five- and ten-g muscle samples from horses experimentally infected with *T. spiralis* were combined with 99-, 95- and 90-g, respectively, of known negative horse tissue to create a 100-g sample for testing. Sample sizes of five and ten-g were more likely to be positive than one-g samples when larval densities were <3 larvae per g (lpg). All five-g samples containing 1.3 – 2 lpg were detected. This validated DSF procedure was consistently effective at detecting all levels of infection considered capable of establishing human infection.

Lorry Forbes
Canadian Food Inspection Agency
Centre for Food-borne and Animal Parasitology
Saskatoon Laboratory
116 Veterinary Road, S7N2R3 Saskatoon, SK
Canada
Phone: 306-975-5345
e-mail: lforbes@inspection.gc.ca

119. Larva *Trichinella spiralis* comes at 30 grams of meat – "Causative connection" is being processed

Cukic G.[1], M. Zivkovic[2], I. Kocan[3], Nada Djuric[4]

[1]Health Home of Berane,
[2]Health Centar of Berane,
[3]Veterinarian station of Berane,
[4]Health Centar of Loznica

The interest of the owner is ambivalent. It makes a dilemma at him: a) It doesn't deny that the health is the most important for him; and so he accepts meat to be destroyed in that higher interest (when there is a proof of larva TS presence). b) It's not all the same to him when he needs to destroy it, at 'uncertain presence of larva', when he takes the same piece of meat at more vets, and for example, only one of them finds larva TS (when there is no certain proof of larva TS presence).

At low-infested consumable meat by larva TS "dilemma" made by different findings of checked meat – 'should be knowledgeable solved'.

Example 1. In studying Berane area in 2000: the fault "sporadic disease" appeared: we have not identified a significant number of diseased, or at least bigger exposure, that is more consumers of the meat with trichinella; but only – epidemic rest: in that one sub clinical diseased. By this, the form of mass disease – one diseased too. From three heads of animal 17 samples were taken and did not succeed to find larva TS – tested samples were 'negative'. Sub clinical disease is confirmed: by positive TIIF on TS (INEP, Zemun), founding antibodies on TS.

As the disease of this clinical form appeared at 'negative infest founding of TS consumer meat'?

Example 2. With weaker invasion, signs of disease appear in a way that "the most diseased young woman... who every day ate smoked meat which consisted small amount of larva." That year 2001. doctors needed 17 days to detect the disease. It was found additionally, by artificial digestion, that in 100 grammas of consumer's meat were 20 larvas, i.e. in 5 grammas of meat one larva.

Example 3. Person once consumed infest meat in October 2002. "Tolerance of the cause" existed for a long. By biological multiplication of grown parasites it came to gradual increase of the number of movable larva. 'Immanent cause' builds up on 34th day – culmination of the larva in organism suddenly, in shown dialectic of events – gave disease.

Example 4. In period from year 2003. to year 2006. HE service and Health Station Berane was informed 12th times by Veterinarian inspection and that veterinarians found infest of the throat by larva TS. Except one reporting, for domestic pig (imported trichinosis), all other referred to wild pigs.

At 10th December 2003. in veterinary station Berane was given to be tested meat of wild pig that weight around 50 kg. By the method of trichinosis-scopes was found the presence of TS larva!!! Then, by method of artificial digestion the finding checked and intensity of

infest found: only three larva were found within 90 grammas of meat (diaphragm, tongue), i.e. one larva on 30 gm. Meat was destroyed, and to (exposed) consumers as prevention was given Mebendazol.

People are exposed to the influence of larva TS by consuming infested meat. There are two possible results:

1. To create causative connection. Created by ruining the tolerance of the organism: a) creating more and more amounts of larva or / and b) taking new amounts of infested meat.

a) On day 34th disease is created'. Meat was, we assume, 'stronger infested'. It was consumed after thermal treatment and one time. For diagnoses of disease and proof over the existence of the epidemic it was important for us to establish qualitatively infest of consume meat by larva TS. Therefore, immediately artificial digestion has been performed. In 30 gm of found meat it was detected 145 larva's, i.e. 5 larva's per one grammas. The presence of TS was found. Organism tolerated until day 33rd created number of movable larva (their processing), but not the number created on day 34th;

b) with often adding, consuming (for example everyday etc.) more and more amounts of meat of low infest, the number of parasites was raised (on the day of checking: it was low; or after treatment: salted, smoked et.).

2. If, sufficient amount of infested meat was not consumed, the manifested clinical image did not appear– the entering disorder (UP) acted in creation of UV. The exposure existed, but immanent UV did not appear. The health remained (disease did not appear) – the number of grown parasites has been tolerated and by their multiplication 'produced' amounts of movable larva. The first extreme is, UP1: destroying all larva by ordinary preparation of meat with thermal treatment ('well fried meat'). The second one, UP2 raising the amount of larva which, in that higher and higher degree, organism still tolerates; before it is transferred (immanent UV creates); and then after starts 'pathogenesis' with: unapparent or manifested clinical image.

Authors conclusion is that routine methods of checking infest of the consume meat – insecure due to their palliatives. Due to tolerance of movable larva the disease can turn out but does not have to. Due to consuming of same meat greater number of times – there is no the lowest stand of the number of larva that causes disease. And because of low infest, tested meat by standard methods very often shows to be without parasites, that makes difficult to diagnose trichinosis, especially if doctors do not take this possibility into consideration – it happens in mass manifestation of trichinosis the appearance of so called 'iceberg' phenomenon. It is still searching for efficient prevention of trichinosis – it is a problem of XXI century.

120. The Comparative Methods in Parasitic Diagnosis of Trichinelosis

Moldoveanu Daniela

Trichinellosis is a parasitic disease, caused by nematodes of the genus *Trichinella*. This parasitozoonosis, with a social and economical impact in both animals and men. Epidemiological statics indicated that trichinelosis are a cosmopolitan infection characterize by tow principal cycles: a domestic-sinantropical cycle which involve *T. spiralis* and a silvatic cycle which involve both *T. spiralis* and another species of *Trichinella*. In present are knowed eight species: five species with capsule (*T. spiralis, T. nativa T. nelsoni, T. murelli, T. britovi*) and tree species without capsule (*T. pseudospiralis, T. zimbabwensis, T. papuae*). At these species was added another few subspecies. In Romania, in 1868, H.S. Scheiber described first case of human trichinelosis, at Coltea hospital. After 3 months, in same year,was descovered first case of infection in pig. In 1913, Curhanski descovered first pig with trichinelosis in the Bucharest abatoire,at just one month after introduction of compulsory control of pig meat, when was utilised imported trichineloscopes. Subsequent, disease was frequent diagnosticated in another species.

The researches were realized in our laboratory for the presence of Trichinella spiralis by the methods trichineloscopy and artificial digestion. The samples using were to pigs and wild boars. Trichinella larvae were obtained by the artificial digestion method and the number of larvae per gram(LPG) was evoluated. The samples diaphragmatic muscle was examined most frequently but tongue, masseter, intercostal muscles were also examined in some cases. The muscle tissue was artificially digested with pepsin and HCL according to standard procedure. Mean intensity of infection varied considerably amoung positive animals, ranging from 0,5 to 51,1 larvae/g. For the trichinoscopy exam we performed a compressed blade with 28 microscope fields from each samples and we counted all the cysts and larvae of *Trichinella*. This particularity of the morfological aspects is very important in differential diagnosis besides of Sarcocystis sp. calcified cysts. Results were positive for Sarcocystis sp. in every samples analyzed, showing a high prevalence similar to that in domestic animals and wild biological cycles in the same habitat. As regarding for the presence of *Trichinella*, both methods confirmed the presence of Trichinella in the samples examinated. However, trichinellosis represent a very hard public health problem for humans and animals.

Daniela Moldoveanu
Institute for Diagnosis and Animal Health
Department of Parasitology Laboratory
Dr. N. Staicovici street 63, sector 5
0050557 Bucharest Romania
Phone: +402 14 12 99 30
e-mail: moldoveanu.daniela@idah.ro

121. *Trichinella* meat inspection by digestion technique: evaluation of a fluid pepsin formulation

Maddox-Hyttel, Charlotte[1], K. Nöckler,[1] E. Pozio[3], Isabelle Vallée[4], P. Boireau[4]

[1]National Veterinary Institute, Section for Immunology and Parasitology, Technical University of Denmark, Copenhagen V, Denmark
[2]Federal Institute for Risk Assessment (BfR), Berlin, Germany
[3]Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy
[4]JRU BIPAR, AFSSA-LERPAZ, Maisons-Alfort, France

Pepsin powder constitutes a health risk, potentially causing severe allergic reactions to those handling the chemical. A pepsin fluid formulation was produced and tested, first in a preliminary study, then in a ring trial encompassing four European National Reference Laboratories (NRLs). The purpose of each trial was to ascertain and compare the pepsin powder with the pepsin fluid regarding the ability to digest meat and to liberate encapsulated *Trichinella spiralis* larvae. The quality of digestion was furthermore evaluated by assessing the visibility through the digestion fluid and the amount of debris remaining after digestion. At each laboratory, 20 blinded replicate 100 g samples of pork meat containing a known number (0-30 larvae) of encapsulated *T. spiralis* larvae were digested by magnetic stirrer method using either the standard pepsin powder or the pepsin fluid (10 samples each). All laboratories found the pepsin fluid to be of equal efficacy compared to the pepsin powder. Similarly, the laboratories found no difference between the two pepsin formulations with regard to debris remnants or visibility through the digestion fluid. The pepsin fluid may therefore constitute a major improvement of the digestion procedure for the personnel involved.

Supported by TrichiNet / TrichiMed

PhD Charlotte Maddox-Hyttel
National Veterinary Institute
Section for Immunology and Parasitology
Technical University of Denmark
Bülowsvej 27
Post Code: DK-1790 Copenhagen V
Denmark
Phone: +45 72 34 62 13
e-mail: cmh@vet.dtu.dk

122. The Diagnosis of the *Trichinella* larvae with morphopathologically modifications in swine meat

Ioan Cironeanu

In infestations with *Trichinella* sp. larvae in swine, there are cases with morphopathological modifications of the parasite, thus the diagnosis cannot be a precise one by using the usual trichinelloscopic examination, additional examinations being necessary. In this situation, we refer to developing larvae, completely covered by inflammatory infiltrates, cysts with larvae covered by dense inflammatory infiltrates, where the presence of the parasite or of the larva cannot be noticed by the trichinelloscope and, finally, calcified *Trichinella* cysts of various sizes, massively covered by calcium salts, which occult the presence of larvae or unresorbed larvae segments.

If the diagnosis cannot be defined by direct trichinelloscopy, the artificial slow digestion may be used. There are not indicated the methods of hard shaking, because the larvae with incomplete development and also the larvae and calcified cysts are destroyed by the dynamic turbulence of the digestion liquid.

The slow artificial digestion is practically made by the thermostat at 37–39°C for about 20 hours or by kiln at 40 - 42°C for about 5 hours. During the digestion, the peptic solution is gently shaken manually or mechanically, two-three times per hour. The sediment resulting after filtration is examined by the trichinelloscope, which shows the cyst (granuloms – 250/180 microns), their structure being fibrous, of almost spherical shape (or oval), in which there are dead larvae or indigested fragments thereof.

Ioan Cironeanu, Veterinary PhD
Association of Romanian Parasitologists
Secretariat
44 Borsa st; 014178 Bucharest 1
Romania
Phone: 0040212329350
e-mail: val.petrescu@romexpo.org

123. Particular morphopathological aspects of *Trichinella spiralis* cysts in swine meat

Ioan Cironeanu

Along with the cysts development of *Trichinella* sp. in swine meat, the immunitary factors of the parasited organisms come locally into action in order to isolate and destroy the free or encysted larvae. As a result, inflammatory reactions occur which are expressed by an inflammatory infiltrate of variable intensity. The inflammatory reaction is produced around free larvae in course of being incysted or around the cysts. Sometimes the inflammatory infiltrate covers partially or totally the *Trichinella* cyst or penetrate it. This pathological process modifies the classical structure and aspect of the parasite. In such cases, the spirals alter, the larva dies, it is occulted by the infiltrate and cannot be clearly distinguished in the trichinelloscopic examination. After 5 – 6 months from the animal's infestation, calcium salts lay on the devitalised larva, largely or totally covering the parasite. The laying of the calcium salts develop from centre to the sides of the capsule, often filling the whole inner space if it. This process modifies the form and the size of the cyst. Generally, the calcium salt deposits have neat, smooth sides, but sometimes these have accretions. It is worth mentioning that in the domestic pig (*Sus scrofa domestica*), the *Trichinella* sp. cysts calcification is made from the interior to the exterior of the capsule, differently to the ones in dogs or humans, where the calcification goes from the capsule poles to the centre.

Ioan Cironeanu, Veterinary PhD
Association of Romanian Parasitologists
Secretariat
44 Borsa st; 014178 Bucharest 1
Romania
Phone: 0040212329350
e-mail: val.petrescu@romexpo.org

124. Comparison of three digestion methods for detection of encapsulated *Trichinella spiralis* larvae in pork : differences in sensitivity between EU officially approved methods

Vallée, Isabelle[1], B. Durand[2], Pauline Macé[1], K. Nockler[3], Charlotte Maddox-Hyttel[4], J. van der Giessen[5], E. Pozio[6], P Boireau[1]

[1]UMR BIPAR, AFSSA-LERPAZ, Maisons-Alfort, France
[2]Epidemiology Unit, AFSSA-LERPAZ, Maisons-Alfort, France
[3]BfR, 7 Berlin, Germany
[4]Dept for Veterinary Diagnosis and Research, National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark
[5]RIVM Microbiological Laboratory for Health Protection, Bilthoven, Netherlands
[6]Department of Infectious, Parasitic and Immunomediated Diseases, ISS, Rome, Italy

In order to harmonise *Trichinella* spp. detection in pork within EU, it was important to compare the three official methods (Commission regulation 2075/2005) to determine whether they are equivalent or not regarding sensitivity of *Trichinella* spp. muscle larvae detection. A ring trial was thus organised between 5 National References Laboratories (NRLs) with sending of 20 proficiency samples/ NRL/method tested. Proficiency samples contained a perfectly known number of *Trichinella spiralis* capsules (1, 2, 3, 7, 8 or 9) and thus allowed us to define directly the sensitivity of artificial digestion method. Two NRLs used magnetic stirrer method, one NRL worked with magnetic stirrer and stomacher, and 2 NRLs treated samples with trichomatic 35®. The best performance in larvae identification was obtained with the magnetic stirrer method with an average of 91,4% (95% confidence interval (CI): 94,3%-87,7%), whereas with the stomacher and trichomatic 35® the recovery was 89,1% (95% CI: 81,3%-94,4%) and 69,8% (95% CI: 62,8%-76,2%) respectively. No significant difference could be demonstrated between magnetic stirrer and stomacher, whereas trichomatic 35® was significantly less sensitive than the two others. Moreover, for proficiency samples containing more than 1 *T. spiralis* capsule, magnetic stirrer method gave high identification rates (>90%).
A focus on this reference method is now needed to improve *Trichinella* spp. muscle larvae detection within EU countries.

Supported by Trichimed.

Isabelle Vallée, PhD
AFSSA-LERPAZ
Department of UMR BIPAR
23 avenue du Général de Gaulle
94706 France
Phone: 33 1 49 77 28 16
e-mail: ivallee@vet-alfort.fr

125. Proficiency sample testing for detection of *Trichinella* larvae in pork

Noeckler, K., Sabine Reckinger

According to Article 5 of Commission Regulation (EC) No 2075/2005 all personnel involved in the *Trichinella* meat inspection shall regularly attend a quality control programme, where negative and *Trichinella*-positive proficiency samples comprising of different larval densities have to be tested. In this connection, the preparation of appropriate proficiency samples is crucial for evaluation of ring trial results.

A national ring trial on detection of *Trichinella* larvae in pork involving 39 laboratories was performed in 2006. Each laboratory had to test a panel of 10 coded proficiency samples consisting of 4 negative and 3 pairs of *Trichinella*-positive samples (with a mean larval load of 6, 10, and 28 larvae per g, respectively). 1 g of each sample was mixed with 99 g of negative meat for examination by magnetic stirrer method. *Trichinella*-positive proficiency samples were produced from a pig experimentally infected with 40,000 *T. spiralis* larvae (strain ISS 003). The larval density was adjusted by mixing negative and *Trichinella*-positive minced pork. The mean number of larvae (per g of muscle) and the tolerance range (mean plus/minus 2-fold standard deviation) for each larval load were calculated from results obtained by a 8-fold examination of 100 g by artificial digestion.

30 out of 39 laboratories (77%) correctly identified all 10 proficiency samples whereas 9 failed to detect one (5 labs) two (2 labs) or three (2 labs) *Trichinella*-positive samples. Out of 15 false-negative results, 9 (60%), 4 (27%) and 2 (13%) related to samples with a mean larval density of 6, 10, and 28 larvae per g, respectively. In regard to the number of larvae, 132 out of 219 *Trichinella*-positive samples (60%) were correctly accounted, whereas 15 (7%) laid above and 72 (33%) below the tolerance range.

Meat samples for proficiency testing with a calibrated number of *Trichinella* larvae could be easily prepared by mixing Trichinella-positive and –negative minced meat, and determination of mean larval number and tolerance range for larval burden was a useful tool for evaluation of ring trial results. Most labs correctly identified Trichinella-positive and –negative samples. However, the exact quantification of larvae in the *Trichinella*-positive samples proved to be more difficult. Therefore, personnel should regularly be trained on proper performance of detection method, knowledge on larval shape, and recognition of possible error sources.

The study was supported by the 6th Research Framework Programme of the European Commission within the Network of Excellence "Med-Vet-Net" (WP TrichiMed).

Karsten Noeckler, PhD.
Federal Institute for Risk Assessment (BfR)
Department of Biological Safety
Diedersdorfer Weg 1, 12277 Berlin
Diedersdorfer Weg 1, 12277 Berlin Germany
Phone: +4930 84 12 20 53
e-mail: karsten.noeckler@bfr.bund.de
Web: www.bfr.bund.de

126. International comparative study to detect anti-*Trichinella* IgG in swine sera

Angeles Gomez Morales, Maria[1], Alessandra Ludovisi[1], B. Liseo[1], F. Pecoraro[2], E. Pozio[1]

[1]Istituto Superiore di Sanita, Rome, Italy
[2]Centro Grandi Apparecchiature Scientifiche, University of Padua, Italy

A ring trial was organised among European (#19) and extra European (#2) laboratories to evaluate the sensitivity, specificity, reproducibility and robustness of an ELISA to detect anti-Trichinella IgG in pig sera. A 96-well microtiter ELISA plate, anti-swine IgG goat antibodies peroxidase labelled, excretory/secretory antigens produced at the CRLP, control (#2 neg.; #6 pos.) and "unknown" (#10) serum samples, and a detailed protocol were provide by the CRLP to participating labs. Laboratories which did not follow the established protocol should write any change. Nine and 14 labs performed the test without (group A) or with (group B) protocol modifications, respectively. From group A, 3 labs got 1, 2 and 8 false negatives. From group B, 6 labs got 1, 2, 2, 14, 3 and 1 false negatives. Using the ROC analysis, the highest sensitivity (97.54%) and specificity (96.84%) was reached setting the cut-off at 18%. A normal mixed effect model was used in which the serum and the lab have been considered random effects, while serum dilutions and protocol effects were considered fix. The goal of this model was to know if the percentage of "true positive" and "true negative" sera might depends on the above effects. The variability due to the serum samples was greater than the variability of the lab, only one laboratory behaved substantially different from the other labs. There was also an important effect of the protocol in the variability of the test results for positive sera and in a lesser extend for negative sera.

Maria Angeles Gomez Morales, PhD
Istituto Superiore di Sanita
Department of Infectious, Parasitic and Immunomediated Diseases
viale Regina Elena 299
00161 Rome, Italy
Phone: +39 06 4990 3379
e-mail: mariaangeles.gomezmorales@iss.it
Web: www.iss.it/crlp/

127. Validation of the PrioCHECK® *Trichinella* Ab ELISA for the detection of porcine antibodies against *Trichinella* spp.

Frey, C.[1], M.E. Schuppers[2], P. Buholzer[3], R. Beck[4], A. Marinculić[4], A.J. Raeber[3], B. Gottstein[1]

[1]Vetsuisse Faculty, University of Bern, Institute of Parasitology, Bern, Switzerland National Reference Laboratory for Trichinellosis in Switzerland
[2]SAFOSO, Bern, Switzerland
[3]Prionics, Schlieren, Switzerland
[4]Faculty of Veterinary Medicine, University of Zagreb, Croatia

Trichinellosis is a zoonotic disease that is caused by nematode worms of the genus *Trichinella*. *Trichinella* spp. maintain their life cycle in many carnivorous and omnivorous animal species, including domestic pigs. In the European Union and Switzerland, pigs are routinely tested at slaughter using the artificial digestion method. Although this method is considered sufficiently sensitive to prevent human clinical disease, it is rather insensitive in samples with low larval densities. At a maturated infection status, serological methods offer higher methodical and thus diagnostic sensitivities. Therefore, lower larval densities are anticipated to be more reliably serodiagnosed than detected upon artificial digestion technique. Consequently, a serological approach could be very suitable as a tool for surveillance to demonstrate freedom from infection. In the present validation study, the diagnostic performance of a newly developed PrioCHECK® *Trichinella* Ab was assessed at the Swiss reference laboratory for trichinellosis. The test procedure was carried out according to the manufacturers recommendations.

The diagnostic sensitivity of the ELISA was determined by testing 95 meat juice samples collected from naturally infected pigs originating from different countries endemic for *T. spiralis* (infection intensities ranging from 350 down to 0.025 larvae per gram muscle), and from pigs experimentally infected with *T. spiralis, T. britovi* or *T. pseudospiralis*. The PrioCHECK® Trichinella Ab ELISA was able to detect trichinellosis in pigs infected with larval loads as low as 0.025 larvae per gram muscle. Ninety-three out of 95 samples were seropositive, yielding 97.9%. It has to be noted that the only two ELISA-negative sera were also negative in a Westernblot used as a gold standard in the serological part of the study. The specificity of the ELISA was determined by a two-step-approach: 800 negative meat juice samples were collected at different Swiss slaughterhouses and represented different pig populations such as fattening

pigs and breeding sows, as well as different production sites, e.g. closed versus free ranging pigs. The status of these samples with regard to *Trichinella* infection was determined by artificial digestion of at least 1 g of diaphragm. Furthermore, samples obtained from pigs with coprologically proven nematode infections including *Ascaris, Trichuris, Hyostrongylus* and *Strongyloides* (n = 74) were included in the specificity study. All samples together (n = 874) yielded negative findings in the PrioCHECK® Trichinella Ab ELISA (specificity = 100%). Conclusively, this commercial assay represents a valuable tool for monitoring, surveillance and certification purposes as suggested in the regulation EC 2025/2005.

Caroline Frey, DVM
Institute of Parasitology, University of Bern
Vetsuisse Faculty
Länggassstrasse 122
3012 Bern
Switzerland
Phone: +41 31 631 24 75
e-mail: caroline.frey@ipa.unibe.ch

128. A novel method for serological monitoring of *Trichinella*-free pig herds

Maassen, Kitty[1], Els Broens[1], R. Achterberg[1], M. Swanenburg[1], S. Haughey[2], Daphne Areskoug[3], P. Bjurling[3]

[1]Animal Sciences Group, Wageningen University and Research Centre, Lelystad, The Netherlands
[2]Xenosense Ltd., Queen\'s Road, Queen\'s Island, Belfast, Northern Ireland
3 GE Health Care, Uppsala, Sweden

A new 2006 EU legislation concerning meat inspection for *Trichinella* spp. offers new opportunities to monitor *Trichinella*-free pig herds using serological methods. In the Netherlands, *Trichinella* is absent in commercial pig farming, and serological monitoring might replace individual carcass control for herds fulfilling the criteria of *Trichinella*-free herds. To detect antibodies against *Trichinella* a Surface Plasmon Resonance (SPR) assay was developed for the commercial available Biacore Q system and a prototype Biacore system designed for high throughput purposes. These two SPR biosensors are developed for the Food Industry. The systems are fully automated and samples are analyzed within minutes. In the assay, ES antigen, is immobilized on a chip. The porcine serum sample is injected and will flow over the chip. When antibodies against *Trichinella* are present they will bind to the ES-antigen yielding a response signal in the instrument. The response is directly proportional to the change of mass on the surface and is measured in real time. The complete assay consists of 2 parts. First a screening assay is carried out; reacting samples can be confirmed in a second step by enhancement. Validation data using negative field samples collected in the Netherlands, positive field samples from two endemic areas and samples from experimental infected swine suggest that the assay is suitable for monitoring Trichinella-free herds as proposed by the EU.

Kitty Maassen, PhD
Animal Sciences Group
Department of Infectious Diseases
P.O. Box 65
8200 AB The Netherlands
Phone: 0031320238084
e-mail: kitty.maassen@wur.nl

129. Diagnostic test evaluation for the surveillance of *Trichinella* infections in pigs - a novel interplay between epidemiology, diagnostics and policy makers

Dörte Döpfer[1], Kitty Maassen[1], R.P. Achterberg1 , J. van der Giessen[2], M. Foinville[2], P. Teunis[2], W. Buist[1], Viviana Molina[3], E. Guarnera[3], M. Rodriguez[3], S. Krivokapich[3], M. Rodriguez[3], J.L. Peralta[4], H. Trabattoni[4], E. Larrieu[5], B. Engel1[1]

[1]Animals Sciences Group of Wageningen UR, Lelystad, The Netherlands
[2]National Institute for Public Health and the Environment, Bilthoven, The Netherlands
[3]Department of Parasitology, National Institute of Infectious Diseases, ANLIS, Buenos Aires, Argentina
[4]Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina
[5]Secretaria de Estado de Salud, Viedma, Rio Negro, Argentina

Trichinellosis is included in the EU white paper on food safety (EC Zoonosis Directive) and the costs for mandatory routine meat inspection of pigs, horses and game animals for *Trichinella* in the EU is estimated to 570 million Euro annually. A new EU directive concerning meat inspection for Trichinella spp. of 2006 offers new opportunities to monitor Trichinella-free herds using serological methods. In the Netherlands, Trichinellosis is absent in industrialised pig farming, and serological monitoring might replace individual carcass control for those herds fulfilling the criteria of *Trichinella*-free herds.

To evaluate the usefulness of serological tests applied to monitor *Trichinella* free herds, Bayesian methodology will be used to estimate the diagnostic test parameters: sensitivity, specificity and prevalence in the absence of a Gold Standard test. In the absence of Dutch serum samples for positive pigs, it is discussed how the diagnostic test parameters derived from panels of sera originating from regions with endemic Trichinellosis in Argentina and Croatia can estimate the test parameters for six different tests.

The diagnostic test parameters from the imperfect serological tests under validation together with prior knowledge about the historically recorded infection status of farms will be used to set up a surveillance system. The surveillance system has to guarantee freedom-of-infection to humans while using an imperfect serological test in a low prevalence population.

130. Development of a capture ELISA for early diagnosis of pig trichinellosis

Lacour, Sandrine[1], Isabelle Vallee[1], F. Serrano[2], K. Noeckler[3], Danielle Lerhun[1], Liu Mingyuan[4], P. Boireau[1]

[1]UMR BIPAR, AFSSA-LERPAZ, Maisons-Alfort, France
[2]UEX, Avda de la Universidad, Caceres, Spain
[3]BfR, Diedersdorfer Weg 1 – D12277 Berlin, Germany
[4]Jilin University, Changchun, PR China

The new EU regulation for pig *Trichinella* infection survey (EC 2075/2005) introduced the serology as a system to control *Trichinella* free herd. A strategy to improve the diagnosis of pig trichinellosis was based on the detection of early circulating antigens of *Trichinella*.

One objective of the TRICHIPORSE contract was to develop an ELISA test capable to fix *Trichinella* antigens before the emerging of specific antibodies. It was expected that this test would detect *Trichinella* infected animals as soon as 1-3 weeks after infection. The development of a capture ELISA was based on 2 monoclonal antibodies with high affinity for epitope specific to the invasive stage of *Trichinella* (NBL).

Since the first results obtained on pig serum were not conclusive, pig muscles were evaluated following special treatments to release *Trichinella* antigens (sonication, rybolysation). A specific signal was obtained from pigs infected by 20,000 *Trichinella spiralis* ML and slaughtered 5, 12, 15, 20 and 60 dpi with a maximum of intensity at 20 dpi. Moreover, highly infected animals by *Trichinella* spp. also gave a good result at 60 dpi. However, this test showed a low sensitivity to moderate-low infections of *Trichinella* spp. The validation step using a group of negative pigs from the field demonstrated the specificity of the test.

In conclusion, the developed capture ELISA allows a high detection of *Trichinella* antigens in muscle sample the first 1-3 weeks after infection, even at 5 dpi.

Sandrine LACOUR, PhD
AFSSA-LERPAZ
Department of UMR BIPAR
23 avenue du Général de Gaulle
94706 Maisons-Alfort France
Phone: 33 1 49 77 46 55
e-mail: s.lacour@afssa.fr

131. Diagnosis of Trichinellosis in pigs by ELISA test with three types of antigens of *Trichinella spiralis*

Ribicich, Mabel[1], H.R. Gamble[2], J. Bolpe[3], I. Sommerfelt[1], S. Mundo[1], N. Cardillo[1], C. San Martin[4], E. Vizio[1], P. Borrás[1], A. Franco[1], A. Rosa[1]

[1]Facultad de Ciencias Veterinarias. UBA
[2]National Research Council. USA
[3]Ministerio de Salud, División de Zoonosis Rurales, Provincia de Buenos Aires
[4]SENASA

In Argentina, many pigs are raised outdoors and *Trichinella* infection is endemic. The tradition of preparing and eating raw pork is common in rural areas of the country and this practice has resulted in outbreaks of human trichinellosis. For these reasons it is necessary to have available effective detection methods for *Trichinella* infection in pigs. In this report, 21 pig farms were selected for study including some using total and partial confinement management, some with pigs raised outdoors, and some with pigs raised under poor hygienic and sanitary conditions. A total of 3224 muscle samples were collected from pigs raised on these farms and these muscle samples were used to determine the presence of *T. spiralis* larvae by artificial digestion (DAR). Serum samples from these same 3224 pigs were tested for antibodies to *T. spiralis* using three ELISA tests: 1) a laboratory ELISA using *T. spiralis* excretory-secretory (E/S) antigens; 2) a Microwell ELISA test kit (SafePath Laboratories St.Paul, MN 55108 USA) also using E/S antigens; and 3) a Glycan Microwell ELISA test kit (SafePath Laboratories Carlsbad, CA 92008 USA) using tyvelose antigen. The laboratory ELISA, the Microwell ELISA and the Glycan Microwell ELISA had sensitivities and specificities of 100% and 100%, 98.3% and 100%, and 98.3% and 100% respectively when compared with results of artificial digestion testing. Results obtained from these three tests were not significantly different ($p \geq 0.05\%$) and the correlation between DAR and the ELISA test was 100% in all three cases. Assuring that pigs are free from *Trichinella* infection is a priority for the pork processing industry in Argentina. In the present study, we have described the performance of several ELISA tests that can be used to detect infected pigs on farms where risk of exposure of *Trichinella spiralis* has been found. Permanent monitoring systems performed in pig establishments, including the use of serology tests, would help to reduce or eliminate the risk for human exposure to pork from *Trichinella* infected pigs.

Mabel Ribicich, Dra, Professor
Faculty of Veterinary University of Buenos Aires
Department of Parasitologia y Enfermedades Parasitarias
Chorroarin 280
1427 CWO Argentina
Phone: +5411 45 80 28 20
e-mail: mribicich@fvet.uba.ar

132. Evaluation of three immunoserological techniques in the detection of porcine trichinellosis

Venturiello, Stella Maris, G.G. Nuńez, Marcela Calcagno, Susana N. Costantino

Three methods used for the detection of porcine trichinellosis (immunofluorescence, IF, EIA and western blot, WB) were compared. Three groups of animals were analysed: 1: pigs with parasite burdens (PB) <1 ML/g (n:18), 2: pigs with PB >2 ML/g (n:23), and 3: animals raised in farms subjected to artificial digestion (AD) of 5 g (n:55). Groups 1 and 2 belonged to outbreaks and analysed by AD of more than 30 g. The detection percentages in pigs'sera with the lower PB were IF:100%, EIA:72% and WB:50%, and serologically positive by 2-3 techniques a 83% of the animals. In pigs with a higher PB the detection percentage was similar for IF and EIA (100%vs91% respectively) and for WB:61%, and serologically positive by 2-3 techniques a 96%. The group 3 had similar percentages for the 3 techniques (IF:27%, EIA:25% and WB:38%) and serologically positive by 2-3 techniques a 22%. Two animals were AD-positive with PB of 0.33 and 2.4 ML/g presenting IF and WB or IF, EIA and WB respectively. Results indicate that: 1-the sensitivity (S)of each technique depends on the PB, always being IF>EIA>WB. For low PB, the decrease in the S is more pronounced for the EIA. Although the WB has a low S, the presence of the specific bands for *T. spiralis* makes it a confirmatory tool. 2-Considering that more than 83% of the animals parasitologically positive had 2 or 3 positive serological techniques, pigs negative by the officially recommended AD but positive by two serological techniques must be regarded as infected pigs.

Stella Maris Venturiello, Ph.D., Proffessor, Reseacher
Chair of Immunology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires-Humoral Studies Institute (IDEHU-CONICET)
Department of Microbiology, Immunology and Biotechnology
Junin 956 4ş floor
1113 Buenos Aires Argentina
Phone: 54-011-4964-8259/60
e-mail: sventuri@ffyb.uba.ar

133. Emergence of the trematode *Alaria alata* in wild boar in France

Vallée Isabelle, H. Ferté, B. Polack, Véronique Lainé-Prade, Pauline Macé, A. Heckmann, P. Boireau

During wild boar meat inspection for *Trichinella* spp detection, trematode larvae were identified in the digestion fluid after muscle digestion. Morphological and molecular typing analysis confirmed the species as *Alaria alata*. Epidemiological studies identified the location where wild boars were infected. This area was within a hunting park with a pond where frogs were proliferating. Since few publications described *A. alata* as zoonotic, carcasses were destroyed or sent for well-cooked preparations with a significant markdown value. Experiments on freezing resistance were performed in order to kill the parasite in meat. Pieces of 100g of muscle were frozen at -18°C for several days. At 5th day, one alive larva was identified but over 5 days no larvae were found in muscle. In order to identify the tissue location of *A. alata*, a mouse was infected per os with 6 larvae. Unfortunately, none of the 6 larvae were identified after 10 days of infection. After comparison of the larvae length and the size of the seave mesh used for *Trichinella* control, it appeared that *A. alata* could not easily pass through suggesting that the size of the mesh was not convenient for *A. alata* recovery. In conclusion, this parasite is difficult to identify in meat due to its non-specific location within the host and its size, which retain it on the surface of the seave. The lacks of knowledge on this parasite and on the zoonotic feature render difficult its control during meat inspection and the prevention regarding human health.

Isabelle Vallée, PhD
AFSSA-LERPAZ UMR BIPAR
23 avenue du Général de Gaulle
94706 Maisons-Alfort
France
Phone: 33 1 49 77 28 16
e-mail: ivallee@vet-alfort.fr

134. A new system for high resolution genotyping of *Trichinella* isolates

Rosenthal, B., G. La Rosa, E. Pozio, D. Zarlenga, D. Dunams

Previous analyses of genetic variation have enabled the differential diagnosis of species of *Trichinella* and have provided valuable insights concerning the temporal and geographic context of their evolutionary differentiation. Tracing their transmission, and detailing their particular distributions in wildlife and domesticated host species, would be more easily accomplished with heritable markers that varied to a greater extent among the individuals of a given species. We therefore have developed a system of variable microsatellite markers that allow more precise quantification of the diversity of particular populations and the extent of gene flow among them. Genotypes are achieved with high reproducibility from small pools of larval parasites using robust PCR amplification assays.

Benjamin Rosenthal, SD
Agricultural Research Service, US Department of Agriculture
Department of Animal Parasitic Disease Laboratory
Beltsville Agricultural Research Center
20705 Beltsville MD USA
Phone: 1 301 504 5408
e-mail: benjamin.rosenthal@ars.usda.gov
Web: http://www.ars.usda.gov/pandp/people/people.htm?personid=12052

135.

Trichinella - free Croatian swine is wanted. Is that possible?

Marinculić, A.[1] , Beck,R.[1], Gašpar, B.[2], Brstilo, M.[2]

[1]Faculty of Vetrerinary Medicine University of Zagreb, Zagreb
[2]Ministry of Agriculture, Forestry and Water Resources of Republic of Croatia, Zagreb

After 10 years of the Governmental Control Program there is now growing confidence that the whole Croatia is not facing anymore a higher risk of human outbreaks as in the past. Ten years intensive activities funded by the Government involved different aspects of monitoring and control measures of the disease. The highest progress within the erradication program was achieved through continuous rodent control in all sites where infected pigs were detected, prompt disposal of infected swine carcasses and compensation to owners of disposed pigs. Today, despite 200 infected backyard pigs, the goal of substantious erradication of swine trichinellosis in Croatia is still within reach. Recent Croatian Trichinella Surveillance Report of just few foci indicated that all that is left towards ending the number of infected swine is just a "clean-up job". Something similar was successfully performed in 2000. After the harmless disposal of all pigs from incriminated backyards the incidence of trichinellosis rapidly decreased. The „ultimate fight" now is to eliminate the last foci of infection, which is suspected to be persisting in Osjecko Baranjska County in the border area of Hungary. It can be stressed that at this time, few years remain before joining the EU and there is no known technical reason why eradication of trichinellosis in swine in Croatia should not be achieved.

136. History of the trichinellosis prevention program in Nunavik, Quebec, Canada

Simard M.[1], J.F. Proulx[2]

[1]Nunavik Research Center , Makivik Corporation, Kuujjuaq, Quebec, Canada, J0M 1C0.
[2]Public Health Department. Nunavik Regional Board of Health and Social Services, Kuujjuaq. Quebec, Canada. J0M 1C0.

Trichinellosis is a mandatory reportable disease in Quebec. In Nunavik (northern Quebec), thirteen outbreaks involving 92 confirmed cases occurred between 1982 and July 2006. Ninety-two percent of those cases were due to consumption of raw walrus meat. To prevent such outbreaks and sustain the hunt, the Nunavik Public Health Department, the Nunavik Research Centre (NRC), the Kativik Regional Government (Hunter Support Program), municipalities and hunters, joined forces to create, in 1992, the Nunavik Trichinellosis Prevention Program. The Canadian Food Inspection Agency also collaborated with NRC to develop a validated digestive method specific to walrus meat. After a relatively slow start and the occurrence of outbreaks in non-or partially participating communities, the program is well in place in all hunting municipalities since 2000. Since then, only black bear meat (4 linked cases) and imported unscreened walrus meat (2 linked cases) have been documented as sources of Trichinella infections in the region. The Nunavik Research Centre coordinates the walrus sampling and provides a 24 hour diagnostic service. The Nunavik population is kept aware of the disease and the prevention program through communications on FM radio, distributions of brochures, diverse General Annual Meetings and Annual Reports.

Manon Simard, Wildlife parasitologist
Nunavik Reseach Centre, Makivik Corporation
Renewable Ressources Department
P.O. Box 179
J0M 1C0 Canada
Phone: 819)-964-2925 ext 259
e-mail: m_simard@makivik.org

137. Sero-surveillance for *Trichinella* infections in animal populations using field sera from endemic and non-endemic areas as a reference

van der Giessen, J.[1], M. Fonville[1], D. Dopfer[2], Kitty Maassen[2], Ineke Eijck[2], P. Teunis[3]

[1]National Institute of Public Health and the Environment, The Netherlands
[2]Animal Sciences Group, Edelhertweg 15, 8219 PH, Lelystad, The Netherlands
[3]Dept. IMA, National Institute of Public Health and the Environment

Modern meat production methods have rendered pork in Western European countries virtually free from *Trichinella*. Monitoring for *Trichinella* remains necessary because *Trichinella sprialis* in meat for human consumption is an important concern for public health. Traditional tests based on digestion of muscle tissue are laborious and expensive for the very large scale use that is necessary for the high production volumes of pork in Europe. We studied serology as an alternative means of monitoring for *Trichinella* in pigs and to be considered for certifying *Trichinella* free herds. Indoor industrialised raised fattening pigs in the Netherlands are used as a negative reference cohort. A positive cohort is not available but we show how sera from an endemic region in Argentina can be used to model a plausible distribution of OD levels in positive sera, employing the difference between the endemic sera and the negative Dutch sera. We describe a method for correcting for variation among testing sera by means of ELISA plates by employing on plate reference sera, and demonstrate how to apply these corrections to a collection of test sera from pig farms.
The thus obtained positive and negative reference distributions can be used to estimate fractions true and false positives, necessary for defining appropriate cutoffs. Based on this analysis, the serological test is shown to lack the predictive power required for its large scale deployment. The properties of the serological test are also compared to the conventional digestion assay, which is less sensitive but has higher specificity. The study was supported by the Inspectorate of Health Protection and Veterinary Public Health, Food and Consumer Product Safety Authority (VWA), the Netherlands

Joke van der Giessen, PhD
National institute of Public Health and the Environment
Department of LZO
Antonie van Leeuwenhoeklaan 9/P.O.Box 1
3721 MA Bilthoven Netherlands
Phone: +313 02 74 39 26
e-mail: joke.van.der.giessen@rivm.nl

138.

Transmission risk of human trichinellosis

van der Giessen, J.[1], M. Fonville[1], P. Teunis[2], Isabelle Vallee[3], P. Boireau[3], Katsuhisa Takumi[1]

[1]National institute of Public Health and the Environment, Department of LZO
[2] Dept. IMA, National Institute of Public Health and the Environment, Bilthoven, Netherlands
[3]JRU BIPAR, AFSSA LERPAZ, Maisons-Alfort, France

Trichinella is a major food-borne parasitic pathogen causing hundreds of infections in humans within Europe each year, and is listed in the EU Zoonoses Directive 2003/99/EC. Foxes and wild boars are the main animal reservoir species in Europe. Also small rodent species may be important as reservoir animals. Transmission of *Trichinella* species between wildlife and the risk it poses to humans via consumption of contaminated pork meat has not been quantified. One pathway by which human trichinellosis can occur is rat - swine - human route. To evaluate transmission risk of this pathway experimental *T. spiralis* infection was performed with doses as few as 10 parasites and the data set was analysed using a newly developed dose response model that describes lpg. Experimental *T. spiralis* infection in swine was analyzed in a similar way. Furthermore six outbreaks of human trichinellosis were analysed for dose response. Risk of human trichinellosis via rat – swine – human pathway was simulated by Monte Carlo. A pair of female and male parasites representing the lowest pressure from the environment led to the probability of human trichinellosis equal to 8% via the pathway. Therefore, low infection pressure from wildlife presents a relatively high risk of human trichinellosis via consumption of contaminated pork meat. Hundred percent of rats were infected with only 10 parasites and their high susceptibility indicates that rats are important in the transmission.

The study was supported by the 6th Research Framework Programme of the European Commission within the Network of Excellence "Med-Vet-Net" (WP TrichiMed) and the Inspectorate of Health Protection and Veterinary Public Health, Food and Consumer Product Safety Authority (VWA), the Netherlands.

Joke van der Giessen, Dr.
National institute of Public Health and the Environment
Department of LZO
Antonie van Leeuwenhoeklaan 9/P.O.Box 1
3721 MA Bilthoven Netherlands
Phone: +313 02 74 39 26
e-mail: joke.van.der.giessen@rivm.nl

139. Assessment of swine farms in Greece in relation to the risk of exposure of pigs to *Trichinella*

Theodoropoulos, G., Helen Theodoropoulou, G. Skopelitis, K. Benardis

Agricultural University of Athens, Faculty of Animal Science, Department of Anatomy and Physiology of Farm Animals, Athens, Greece

Commission Regulation (EC) No 2075/2005 of 5 December 2005 lays down detailed conditions for *Trichinella*-free farms. The objectives of the present study were 1) to assess the current state of swine farms in Greece in relation to the risk of exposure of pigs to *Trichinella* as a first step towards certification of *Trichinella*-free farms in Greece and, 2) to identify a profile and characteristics of swine farms and farmers which can be used to formulate appropriate measures to promote good production practices for reducing risk of exposure of pigs to *Trichinella*. A total of 70 swine farms were inspected using audit components similar to those used by the U.S. Trichinae Certification Programme. Only four farms (5.7%) complied with all the criteria used for assessing the good production practices for reducing risk of exposure of pigs to *Trichinella*. A common characteristic of these four farms was that they produce their own feed while all the other farms that did not meet the criteria purchase feed. The most common issues among the farms that did not meet audit criteria in the present study were related to proper rodent control and accessibility of other animals in the feed storage area and the facility. In addition, significant differentiating characteristic for the farms not meeting the criteria was their small (<150 sows) or medium (151-300 sows) size, while for the farmers it was their failure to meet compulsory education requirements. These characteristics, which are linked to poor infrastructure and reluctance for improvements, respectively, will be difficulties that must be dealt with in the promotion of good production practices for *Trichinella*-free farms in Greece.

PhD. Georgios Theodoropoulos
Agricultural University of Athens
Faculty of Animal Science
Department of Anatomy and Physiology of Famr Animals
75 Iera Odos, 11855 Athens, Greece
Phone: +30 210 5294387
e-mail: gtheo@aua.gr

140. Evaluation of the risk of transmision of *Trichinella spiralis* in pork production systems of Argentina

Ribicich Mabel[1], R.H. Gamble.[2], J. Bolpe.[1,3], Irma Sommerfelt[1], Natalia Cardillo[1], Exequiel Scialffa[3], R. Gimenez[1], Mariana Pasqualetti[1], Graciela Pascual[1]; A. Franco[1], Adriana Rosa[1]

[1]Facultad de Ciencias Veterinarias. UBA
[2]National Research Council. USA
[3]Ministerio de Salud, Division de Zoonosis Rurales, Provincia de Buenos Aires

To control the increasing incidence of human trichinellosis in Argentina, methods for the detection of *Trichinella* infected pigs at slaughterhouses have undergone important changes. Recently, there has been interest in programs that provide documentation of pork production practices that minimize the risk of exposure of pigs to *T. spiralis*. In this report, 21 pig farms were selected for study including some using total and partial confinement management, some with pigs raised outdoors, and some with pigs raised under poor hygienic and sanitary conditions. A total of 3224 muscle samples were collected from pigs raised on these farms and these muscle samples were used to determine the presence of *T. spiralis* larvae by artificial digestion (DAR). Serum samples from these same 3224 pigs were tested for antibodies to T. spiralis by ELISA using an excretory-secretory (E/S) antigen. For each farm where samples were collected, a questionnaire was completed summarizing information about management factors and this information was analyzed relative to knowledge of risk factors for exposure of *T. spiralis*. Based on testing results, pigs whose origin was not known were 3.2 times more likely to be *Trichinella* positive than pigs from known farms. Pigs raised outdoors were also more likely to be infected than pigs raised in total or partial confinement ($p \leq 0.05$). Pigs fed waste products containing meat were 12.5 times more likely to be infected than pigs not fed waste containing meat ($p < 0.01$). The role played by rats in transmission of *Trichinella* is still obscure; however, on farms with evidence of wild animals and access to wildlife carcasses, the prevalence of *Trichinella* infection in pigs was significantly higher. All pigs raised under good hygienic and sanitary conditions were negative for Trichinella infection by both DAR and ELISA. Raising pigs free from Trichinella infection is a priority for the pork processing industry in Argentina. The implementation of good management practices for raising pigs and regular monitoring of pigs, by ELISA and DAR, during production and slaughter will help to reduce the risk of human exposure to this parasite.

Mabel Ribicich, PhD, Professor
Faculty of Veterinary University of Buenos Aires
Department of Parasitologia y Enfermedades Parasitarias
Chorroarin 280
1427 CWO Argentina
Phone: +5411 45 80 28 20
e-mail: mribicich@fvet.uba.ar

141. Accession of UE and trichinellosis in Poland

Różycki, M., J. Osek

National Veterinary Research Institute
Pulawy, Poland

In 1991 the Association Agreement was signed between Poland and the European Communities and their Member States. In order to become a fully-fledged EU member Poland was required to carry out reforms in many sectors of the Polish economy and health care system to harmonise them with the rules effective in the EU. The harmonisation process has taken place since the effective date of the commercial part of the European Treaty in 1992. Facing the membership negotiations it was necessary to intensify, better systematise and co-ordinate the harmonisation processes. A tool for implementing the above actions was the National Programme for the Adoption of the Acquis (NPAA). In June 2002, the Ministry of Health took over the functions of co-ordinator of activities relating to food safety previously undertaken by different ministries and central institutions. The Food and Nutrition Institute together with National Veterinary Research Institute developed a Food Safety Strategy (FSS). The FSS requires legislative changes pertaining to food safety regulation and powers of official food control bodies. Review of 17 parliamentary acts regulating food safety issues showed that four required further amendment despite the fact that they were enacted in 2001. This was due to the fact that they were not fully harmonised with new 2002 EC directives and regulations as well as amendments to some EU legal acts. The HACCP principles were implemented before accession. The upgrading of food - processing establishments has been progressing well, particularly in the meat sector. A significant number of establishments evaluated, some with little prospect of meeting the relevant acquis have been closed. The first transition phase (1989-1992) showed a fast growth in the number of private processing plants. Since 1992 the output in the industrial plant category has been growing faster than in local processing plants. The position of small local companies was deteriorating, since they were losing competitive advantage they won in the first phase of transition period. Medium-size companies were strengthening their positions pretty fast. The Central Statistical Office (GUS) data showed that there were 30,000 companies operating in the food processing sector. At present there are almost 23,000 small local processing plants (with

employment below 9 workers), 5,600 of enterprises (below 49 workers) and approx. 1,700 of large and medium - size companies that hold almost 70% share in total output of food processing sector. Structural changes fuelled by tough competition, including, among others, surplus of raw material supply over demand, had also impact on major agricultural producers. This led to improvement of agricultural production quality. Foreign direct investments (FDI) in the overall food sector amounted to approx. US $ 5 billion by the end of 2000. Such investments many times opened access to world markets. Capital expenditures in 1990-2000 were above US $ 12 billion. In 70% the funds were utilised to purchase machinery and modern process lines. This allowed to cover a technological gap and to modernise the processing industry quickly. After 1999 the investment rate declined by 20%. In 2000 capital expenditures amounted to US $ 1.2 billion and the share of expenditures for non-production assets also increased (e.g. harmonisation with sanitary requirements).

The production and processing of red meat is one of the largest sections in the Polish economy, since the Polish population spends on meat approximately 10% of total spending (approx. 30% of spending on food). The output of pork and beef accounts for 33% of commodities produced by agriculture and approx. 30% of final products. Poland is 4th in Europe and 6th in the world producer of pork. The transition period influent on the diagnostic of *Trichinella spiralis*. During the transition period were confirmed over 700 cases of pig's trichinellosis. Additionally over 1000 wild bars were infected. It has been observed that until the middle 90-ties the main source of trichinellosis for humans was a consumption of infected pig meat. Since 1995 the wild boars meat occurs as the new main source. The number of outbreaks combined with consumption of pig meat has decrease about 86%, but at the same time the with the boars meat number increase in 10%. The number of infected humans decreases in 50%. The restructuring led to the increase of the size of average herd and improvement of meat quality, and better diagnostic of trichinellosis.

M. Różycki
National Veterinary Research Institute
Pulawy, Poland
mrozycki@piwet.pulawy.pl

142. Detection of trichinellosis in an historically *Trichinella*-free area of Argentina

N. Costantino, Susana, Natalia Sosa, Marcela Calcagno, María Analí Forastiero, S.P. Farabello, María Rosalba Taus, Stella Maris Venturiello

The aim of the present work was to determine the presence of human and porcine trichinellosis in an historically *Trichinella*-free area of Argentina. Blood donors (n:216) and pigs destined for consumption (n:57) were evaluated by immunoserological techniques (ELISA, immunofluorescence and/or western blot). Moreover, 26 out of the 57 animals were parasitologically evaluated by the artificial digestion method. Epidemiological data were collected by means of a questionnaire to evaluate the alimentary habits of the population as well as the raising conditions of swine. Surveys showed that a 98.1% of the individuals (n:212) were habitual consumers of pork in the form of stuffed products acquired mainly at the butcher's. The seroprevalence (positive sera by two or three methodologies) was 8.3% (n:18) for human trichinellosis and 24.5% (n:14) for porcine trichinellosis. *Trichinella* larvae were found in a 7.7% (n:2) of the evaluated animals, with parasite loads of 0.33 muscle larvae (ML)/g and 2.4 ML/g. The isolated ML corresponded to *T. spiralis*. Twelve Trichinella-positive animals were swine raised in deficient conditions. Our studies confirm the existence of human and porcine trichinellosis in an area regarded as *Trichinella*-free and demonstrate that the lack of reports over a long period is not sufficient for an area to be considered as *Trichinella*-free.

Stella Maris Venturiello, Ph.D., Proffessor, Reseacher
Chair of Immunology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires-Humoral Studies Institute (IDEHU-CONICET)
Department of Microbiology, Immunology and Biotechnology
Junin 956 4ş floor
1113 Buenos Aires Argentina
Phone: 54-011-4964-8259/60
e-mail: sventuri@ffyb.uba.ar

143. Seroprevalence of *Trichinella* in slaughter pigs in Kathmandu Valley, Nepal

Sapkota B.S., F. Hörchner, L. Srikitjakarn, M.N. Kyule, M.P.O. Baumann, K. Nöckler

The aim of the present study was to determine the *Trichinella* (T.) seroprevalence in slaughter pigs in Kathmandu Valley, Nepal. In total, 400 sera of pigs were collected from 4 major slaughter slabs and tested for Trichinella antibodies by ELISA using larval excretory–secretory (E/S) antigen. In result, four were positive and one was doubtful, giving a Trichinella seroprevalence of 1% (95% CI: 0.27 - 2.54). In titration, all positive and doubtful sera showed a borderline titer with higher than 1:80. Slaughter pigs were from four major areas of Nepal namely, Kathmandu Valley, eastern Nepal, Terai and adjoining areas of the valley. Positive results were found only in Kathmandu Valley and adjoining areas. There was no significant difference in the prevalence between areas ($p = 0.43$). All four positive sera were from indoor managed pigs. Serological findings indicate the presence of *Trichinella* in Nepal and highlight the need to find out the species and infection sources.

Buddhi Sagar Sapkota, Asst. Professor
Himalayan College of Agricultural Sciences and Technology (HICAST) Gatthaghar, Bhaktapur
Nepal
Department of Veterinary Public Health
Purbanchal University Nepal
P.O.Box: 13233, Kathmandu, Nepal
Phone: +977 16 63 07 62
e-mail: dr_sapkota@hotmail.com
Web: www.hicast.edu.np

144. Medico-legal Implications of the Trichinosis Diagnosis on Animals

Cristea, Gh.[1], D. Marculescu[2], V. Jula[2], Eugenia Cristea[3], Debora Cristea[4], Olimpia Bortas[5]

[1]CSVCA Vulcan, Romania,
[2]Serviciul Medico-legal Hunedoara, Romania
[3]CMI Vulcan, Romania,
[4]Medic rezident cardiologie Timiosara, Romania,
[5]DSVSA Hunedoara, Romania

The rebutal and diminution of the criminality in the fight for human defending against parasitical diseases transmited from animals, together with the safeguarding of the safety and security of the animal source food could be realized only through a sound knowledge of parasitical diseases'diagnosis, rebutal and prevention, especially of trichinosis, and in the conditions when legal initiatives and medico-legal measures are both undertaken which are able to allow the specialized institutions with anticriminal duties.

During the last two decades (1987-2006) in the Jiu Valley were examined 242.504 pigs among whom 3.951 were found infested with *Trichinella spiralis* larvae (1,62%).

The trichinosis diagnosis in animals has a special importance from theoretical, scientific and especially practical perspective. The ‚intra vitam' diagnosis consisting in clinical exam, paraclinical examinations (leucocytary formula determination, intradermo - reaction, ELISA test, flocculence reaction, circumlarvar precipitation, a.s.o.), cervical or lingual biopsic exam, the exam of cremasterian muscles obtained from males gelding has an important role in animals population surveillance but has limitations, is approximately and we must take in account that positive false or negative false reactions can occur and that's why it must be realized with much discrimination.

The post-mortem trichinosis diagnosis on animals has much more safety being more reliable when it is executed with much concientiousness and rigour; trichineloscopical on pressing mount method (compressor) and peptical digestion are universally accepted. The methods have also limitations and their eficiency depends on examinator's level and experience, on the choosen method, on animal specia the muscular tissue comes from and on infestion's intensivity based on body part. These trichinosis diagnosis confirmation exams depends on apparatus, on the way of samples' gathering, on work methods and on the parasite evolution stage during the biological cycle. Based on these facts the diagnosis error can be situated among 1 and

70 %, that's why the diagnosis evaluation, from the medico-legal point of view must be done with much discrimination.

Extens-intensivity of the natural infestation with *Trichinella* sp. in pig, wild boar, rat, bear, horse is maximumwhen are examined the external muscles of the tongue. In mouse, the greatest concentration of *Trichinella* larvas was identified in the lateral-ventral abdominal muscles (the muscles of the flank) and in the maseterian muscles; in foxes the most infested muscles were found the eye's muscles and the forearm's muscles; in dogs - the profound cervical muscles and the forearm's muscles and in the experimental infestation in rabbit, sheep, guinea pig, hare, the most infested muscles were the maseterian ones and in hedgehog the maximum intensivity was found in the forearm's and the omoplat's muscles.

145. Considerations regarding the trichinosis evolution in swine in Hunedoara County, during the 2004-2006 Period

Bortas, Olimpia[1], I. Petrusan[1], D. Marculescu[2], V. Jula[2], Gh. Cristea[3]

[1]D.S.V.S.A. Hunedoara, Romania
[2]Serviciul medico-legal Hunedoara, Romania
[3]C.S.V.C.A. Vulcan, Romania

The extensivity of the trichinosis and the animals' receptivity, especially the pigs' to this disease kept up during the last years in Hunedoara county.

Among the factors that mantained a high incidence of trichinosis in pigs during the last 3 years we mention: unproper condition breeding, precarious zoohygiene, offals from containers or garbage platform animal feeding, surreptitious trade with swine meat from uncontrolled sanitarian-veterinarian sources, lack of sistematic deratization, lack of crematories, lack of sanitary education, poverty, a.s.o.

During this period (2004-2006), 65 animals out of 134.052 pigs were found infested with trichinella sp. larva (0,05%).

In 2004, 39 animals out of 65.638 pigs were found infested (0,06%); in 2005, 8 cases out of 28270 were found infested, representing 0,03%.

The collaboration between different specialists, together with population's active and aware participation, the decision factors' implication and the poverty's elimination will create the possibility of this very damaging disease - trichinosis in human and animals.

146. Swine trichinellosis in one endemic district in Serbia, epidemiology and control

Zivojinović M.[1], Dimitrijević G.[1], Lazić M.[1], Petrović M.[2], Sofronić-Milosavljević Lj.[2]

[1]Veterinary Specialistic Institute "Pozarevac", Pozarevac, Serbia
[2]Institute for the Application of Nuclear Energy "INEP", University of Belgrade, Belgrade, Serbia

In Serbia trichinellosis is re-emerging, endemic and in the same time the main parasite zoonosis that presents serious public health and animal husbandry problem. Human health problem is mainly the consequence of high prevalence of infection in domestic animals, especially swine. Presented epidemiological data were collected for 12 years period (1995-2006), at small private farms located in one endemic district of Serbia (Region of Branicevo). Parasitological examinations indicated that 8.889 (0.57%) animals out of 1.554.262 slaughtered swine were infected. Fluctuations around the mean value for number of infected swine (740.8 ą 275.9), in 6 parishes of above mentioned district, reflected epidemiological situation for each particular year. For monitoring of Trichinella infection prevalence in swine, the serological survey was performed during 2006. Parasite specific antibodies were detected in 15 swine sera (0.16 %), while doubtful ELISA results were obtained in 10 (1.09 %) out of 916 analyzed swine sera. Serological retesting was performed at INEP. All 25 animals, proclaimed as suspected to *Trichinella* infection, were located in all parishes except one (Pozarevac). Parasitological confirmation was performed only in 10 animals (those accessible to veterinarian inspection). Our results point out the importance of monitoring the disease prevalence in aim to achieve better control of trichinellosis. (Projects No 447/2005, 143047).

Milena Zivojinovic, DVM
Veterinary Specialistic Institute Pozarevac,
Contagious diseases of animals and zoonoses
Department of Epizootiology
Dunavska 89
Post Code: 12000 Pozarevac Serbia
Phone: ++381(0)12 554 840
e-mail: povetinst@ptt.yu

147.

Economic impact of trichinellosis in the District of Vukovar

Legen, S.[1], Marinculić, A.[2], Tadić, M.[2], Gašpar, A.[3] Barišić,N.[3], Desnica,B.[4] Mišetić,Ž.[5], Krešić,K.[6]

[1]Veterinary Station, Čakovec
[2]Faculty of Veterinary Medicine University of Zagreb
[3]Ministry of Agriculture Forestry and Water Resources, Zagreb
[4]Clinic for Infectious Diseases „ Fran Mihaljević“, Zagreb
[5]General Hospital, Vinkovci
[6]Institute for Public Health, Vukovarsko srijemska County, Vinkovci

The decision on financing the measures that are being taken to prevent the appearance of trichinellosis among animals and especially people, can be easily made once the cost-benefit analysis has been carried out. The research was done for a one year period in the District of Vukovar. In order to justify the measures financed from the government budget, as well as to implement them further, an analysis of costs caused by trichinellosis as a disease was carried. The following costs financed from the government budget were analysed:

- rodent control in towns, local districts and villages where the presence of the *Trichinella spiralis* parasite was diagnosed after the post-mortem examination of the slaughtered pigs.
- harmless disposal of animal carcasses where the presence of the *Trichinella spiralis* parasite was diagnosed.
- compensation to owners of slaughtered animals where the presence of the parasite was diagnosed.

In 2000, in addition to these costs, the government budget financed buying off of all the pigs that were in contact with the animals that were diagnosed with *Trichinella spiralis* using the method of artificial digestion.

The costs of trichinelloscopy, or artificial digestion, after slaughters in farms and in slaughter houses were also included.

Diagnostics costs, treatment and hospitalisation costs of trichinous patients and sick-leaves were also analysed.

148. *Alaria alata* as a common finding during examination of wild boar meat

Milešević, M.

Mario Veterina Veterinary Clinics, Sisak, Croatia

As a consequence of frequent outbreaks of trichinellosis in pigs the Ministry of Agriculture, Forestry and Water Resources promulgated a decree of obligatory testing of wild boar meat. According to the Croatian Ordinance for the Postslaughter Control of Meat for Human Consumption all wild boars has to be tested by artificial digestion. Oposite to a very low prevalence of *Trichinella*, in a very short time period, diagnostic laboratories reported a high prevalence of mesocercaria *Alaria alata*. Since previous reports of few authors clearly shows that mesocercariae could be harmless for humans the same Ministry proclaimed that the wild boar carcassess has to be properly disposed. It has to be stressed that the highest number of reports came from the laboratory engaged in the examination of wild boars from the District of Moslavina. In order to define the real prevalence of mesocercariae a thorough examination of 54 wild boars killed in the hunting area of river Odra (District of Moslavina) was performed. Totally 30 grams of diaphragm pillars from each animal were digested. Among 54 wild boars even 32 (59%) were found infected. Further epidemiological studies of frogs (Rana esculenta) as potential indirect hosts were also performed. Totally 43 frogs were collected from the same area. The whole thigh muscle of both legs were digested. Out of 43 examined frogs, 18 frogs (48,86%) were found positive with the burden from one 1 to 34 mesocercariae.


PRIONICS


FOSS


SafePath Laboratories


MEDICINSKA TEHNIKA
INEL


OLYMPUS


MED•VET•NET


HAH
HRVATSKA AGENCIJA ZA HRANU


BIACORE
Part of GE Healthcare
www.biacore.com/food