International Commission on Trichinellosis

Jilin University

PROGRAM AND ABSTRACT BOOK

Thirteenth International Conference on Trichinellosis

Changchun, China, 1-6 August, 2011

13th International Conference on Trichinellosis

Changchun, China

1st – 6th August, 2011

ICT13 Website: http://www.ict13.org

Organizer:

International Commission on Trichinellosis
Jilin University

Editors of Abstract Book:

Ray Gamble
Alvin Gajadhar
Dolores Hill
Benjamin Rosenthal
Mingyuan Liu

13th International Conference
on Trichinellosis

ABSTRACT BOOK

Changchun, China
1st – 6th August, 2011

WELCOME ADDRESS

Dear ICT Members, Guests and Friends,

I would like to extend a warm welcome to those attending the 13th International Conference on Trichinellosis on August 1-6, 2011 in Changchun, China. The Local Organizing Team led by Professor Mingyuan Liu has put together an exciting scientific agenda and a very full and interesting social program.

Trichinella remains an important foodborne pathogen in some parts of the world and international trade in many countries relies on programs to test or otherwise assure that pork and other food animals are free from, this parasite. The International Commission on Trichinellosis serves an important role in providing the highest quality scientific information on the biology, systematics, epidemiology, detection, human disease diagnosis and treatment. The science that we provide is widely used in both preventing human exposure and, when necessary, treating human disease.

Trichinella has also served as an excellent model for studies in basic immunology and biochemistry. The knowledge developed from the study of *Trichinella* has been applied broadly to other systems.

As we meet in Changchun, we come together to share our collective knowledge of this most interesting parasite and to look for new opportunities to collaborate on important research topics. I hope you will take full advantage of this wonderful opportunity to interact with your colleagues.

Best wishes for a very successful ICT-13

Ray Gamble, President

COMMITTEES

ICT Executive Committee

- President: Ray Gamble, USA
- Vice president: Albert Marinculić, Croatia
- Secretary general: Christian Kapel, Denmark
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Local Organizing Committee

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ACKNOWLEDGEMENTS

The organizers gratefully acknowledge the support of the following institutions and sponsors for their great contribution:

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PROGRAM

1st August, Monday

13:00 - 15:30 **ICT QA MEETING**
Chair: Ray Gamble, Alvin Gajadhar

16:00 - 17:30 **1st ICT EXECUTIVE COMMITTEE MEETING**
Chair: Ray Gamble

17:30 **WELCOME RECEPTION**

2nd August, Tuesday

08:30 - 09:00 **PRESIDENTIAL ADDRESS**
Chair: Christian Kapel

09:00 - 11:40 **SESSION: LEGISLATION AND FOOD SAFETY**
Chair: Ray Gamble, Alvin Gajadhar

09:00 - 09:25 **Plenary Lecture**
Standardizing Science-Based Control Measures for *Trichinella* in Global Pork Trade
Ray Gamble, Alvin Gajadhar

09:25 - 09:40 Retrospective analysis of proficiency testing results of analysts in laboratories performing *Trichinella* digestion assay
Brad Scandrett

09:40 - 09:55 Towards a standardised surveillance for *Trichinella* in the European Union
Edoardo Pozio

09:55 - 10:10 The *Trichinella* Reference method - interactive CD-ROM
Marleen Claes

10:25 - 10:40 *Trichinella* detection: Identification and statistical evaluation of sources of error in the official reference method
Katharina Riehn

10:40 - 10:55 How safe is the meat inspection system using the artificial digestion of pooled samples: a scenario in a *Trichinella* non endemic region in Europe.
Joke van der Giessen

10:55 - 11:10 Study on the suitability of inactivated *Trichinella spiralis* for proficiency samples
Karsten Nöckler

11:10 - 11:25 Working team for trichinellosis - a systematic approach to prevention, control and eradication of trichinellosis in the Republic of Croatia
Davor Balić

11:25 - 11:40	Differences in the prevalence of *Trichinella* sp. in domesticated and wild boar influence the public-health consequences of insensitivity of diagnosis by trichinelloscopy: a census survey in an endemic region of Romania **Radu Blaga**
11:40 - 12:00	**POSTER EXHIBITION**
13:30 - 15:25	**SESSION: TRICHINELLA IN ANIMALS** **Chair: Edoardo Pozio, Christian Kapel**
13:30 - 13:55	**Plenary Lecture** The opportunistic nature of *Trichinella* - exploitation of new geographies and habitats **Edoardo Pozio**
13:55 - 14:10	Rat's defence against *T. nativa* is multifactorial **Antti Sukura**
14:10 - 14:25	Experimental infection with *Trichinella* T12 in domestic cats **Miriam Mabel Ribicich**
14:25 - 14:40	High prevalence and low level of *Trichinella* infection in domestic pigs of northern and eastern Henan, China **Jing Cui**
14:40 - 14:55	*Trichinella* infection in fox (*Vulpes vulpes*) in Slovenia **Janez Posedi**
14:55 - 15:10	*Trichinella* infection in Mustelidae (Mammalia: Carnivora) from Romania: new host-parasite associations in a highly endemic country **Călin M. GHERMAN**
15:10 - 15:25	*Trichinella* infection in herbivores, do we wish to know more? **Antti Oksanen**
15:40 - 17:05	**SESSION: GENOMICS AND PROTEOMICS** **Chair: Pascal Boireau, Dolores Hill**
15:40 - 16:05	**Plenary Lecture** Inflammatory phenotype of *Trichinella spiralis* nurse cell inferred from transcriptome analysis **Magdalena Dabrowska**
16:05 - 16:20	Molecular cloning and identification of a cystatin gene TsCystatin1 from *Trichinella spiralis* **Baoquan Fu**
16:20 - 16:35	Transcriptome of small regulatory RNAs in the development of the zoonotic parasite *Trichinella spiralis* **Xiaolei Liu**
16:35 - 16:50	Protein changes of *Trichinella spiralis* infective larvae and intestinal epithelial cells after co-culture *in vitro* **Lei Wang**

16:50 - 17:05 Is it possible to define the pattern of *Trichinella* sp. infection for human and pig sera by western blot?
Maria Angeles Gómez Morales

17:15 - 17:35 POSTER EXHIBITION

3rd August, Wednesday

08:30 - 09:55 SESSION: EPIDEMIOLOGY AND CONTROL
Chair: Joke van der Giessen, Albert Marinculić

08:30 - 08:55 **Plenary Lecture**
Current state and control of trichinellosis in endemic countries
Albert Marinculić

08:55 - 09:10 Current status of trichinellosis in Serbia
Ljiljana Sofronic - Milosavljevic

09:10 - 09:25 Characteristics of the Epidemiological Process in the Development of Human Trichinellosis in Brasov County-Romania during 1983-2007
Cristina Elena Dobrescu

09:25 - 09:40 An update on the *Trichinella britovi* focus on the island of Sardinia, Italy
Edoardo Pozio

09:40 - 09:55 Towards a surveillance for *Trichinella* in Slovenia
Golinar Oven Irena

10:10 - 15:15 SESSION: DIAGNOSTICS
Chairs: Karsten Nöckler, Patrizia Rossi, Maria Angeles Gómez Morales

10:10 - 10:35 **Plenary Lecture**
The EU perspective on risk management for trichinellosis
Karsten Nöckler

10:35 - 10:50 **Sponsor**
A novel method for the detection of *Trichinella* in swine meat: *Trichin*-L
Gilles Nespoulous

10:50 - 11:10 **Sponsor**
New artificial digestion assay-An alternative to the current pepsin digestion
Patrik Buholzer

11:10 - 11:20 **Sponsor**
Multispectral direct detection of *Trichinella* larvae on nylon filters
Christian Kapel

11:20 - 11:40 International standardization of *Trichinella* detection methods
Patrizia Rossi

11:40 - 12:00 POSTER EXHIBITION

13:30 - 13:45 Validation of a latex agglutination test for the detection of *Trichinella* infections in pigs
Gianluca Marucci

13:45 - 14:00 Evaluation of a novel bead-based assay for simultaneous determination of specific anti-body responses against *Trichinella spiralis* and *Toxoplasma gondii* in *porcine* serum
Gertie Bokken

14:00 - 14:15 Detection of *Trichinella spiralis* circulating antigens in serum of experimentally infected mice by an IgY-mAb sandwich ELISA
Zhongquan Wang

14:15 - 14:30 Establishment and initial Application of Real-Time PCR Detection Kit for *Trichinella* isolates in muscles
Mingxin Song

14:30 - 14:45 Excretory/secretory biomolecules from *Trichinella spiralis*/*T. britovi* L1 larvae as potential diagnostic markers
Břetislav Koudela

14:45 - 15:00 Characterization of DAF-21/HSP90, an antigen protein from parasitic nematode *Trichinella spiralis*
Yurong Yang

15:00 - 15:15 Production and characterization of monoc-lonal antibodies against a serine protease from newborn stage of *Trichinella spiralis*
Karsten Nöckler

15:30 - 17:40 SESSION: TRICHINELLA BIOLOGY
Chair: Jean Dupouy - Camet, Takahashi Yuzo

15:30 - 15:55 **Plenary Lecture**
Trichinella: What's going on during nurse cell formation
Zhiliang Wu

15:55 - 16:10 Analysis of the effect of the new benzimidazole derivate GNV14, on *Trichinella spiralis* muscle larvae
Lilián Yépez Mulia

16:10 - 16:25 A new formulation of mebendazole in low-substituted hydroxypropylcellulose (L-HPC): improved efficacy against experimental trichinellosis
Francisco Bolas-Fernandez

16:25 - 16:40 TGF-beta and hedgehog signalling pathways in *Trichinella spiralis*
Bernadette Connolly

16:40 - 16:55 Antigen genes display of *Trichinella pseudospiralis* at different development stages
Xiuping Wu

16:55 - 17:10 *Trichinella spiralis* and *Caenorhabditis elegans* display a parallel pattern of thymidylate synthase localization.
Magdalena Dabrowska

17:10 - 17:25 Freezing resistance of *Trichinella* muscle larvae in wild boars experimentally infected
Sandrine A. Lacour

17:25 - 17:40 Serine proteinase inhibitor (Serpin)-A potential good diagnosis target for *Trichinella* late infection
Jianli Yu

17:40 - 18:00 POSTER EXHIBITION

4th August, Thursday

08:30 - 10:10 SESSION: PHYLOGENY
Chair: Dante Zarlenga, Benjamin Rosentha

08:30 - 08:55 **Plenary Lecture**
Sequencing the genome of *Trichinella spiralis*: learning from the past
Dante Zarlenga

08:55 - 09:10 Shared and disparate patterns of developmentally regulated gene expression in two agents of human trichinellosis.
Fei Gao

09:10 - 09:25 Molecular identification of nematode larvae different from those of the *Trichinella* genus detected by muscle digestion
Gianluca Marucci

09:25 - 09:40 *Trichinella spp.* infections in different host species of an endemic district of Serbia
Milena Zivojinovic

09:40 - 09:55 Cross-breedings between *Trichinella* T12 and the other encapsulated *Trichinella* species suggest its reproductive isolation
Edoardo Pozio

09:55 - 10:10 Inferring the history of *Trichinella* diversification and dissemination from analyses of genetic variability: Recent achievements, future potential, and practical limitations
Benjamin Rosenthal

10:25 - 11:45 SESSION: HUMAN TRICHINELLOSIS
Chair: Judith Appleton, Zhiliang Wu

10:25 - 10:50 **Plenary Lecture**
Travel: a new driver for trichinellosis
Jean Dupouy-Camet

10:50 - 11:05 The Worldwide Occurrence and Impact of Human Trichinellosis, 1986 - 2009
Edoardo Pozio

11:05 - 11:20 Clinical Manifestations in Human Trichinellosis - Retrospective Epidemiological Study
Zamfir M. Carmen Anita

11:20 - 11:35 Seroprevalence of *Trichinella* antibodies in blood donors, France
Jean Dupuoy-Camet

11:35 - 11:55 POSTER EXHIBITION

13:30 - 16:20 SESSION: IMMUNOLOGY
Chair: Lilián Yépez Mulia, Fabrizio Bruschi

13:30 - 13:55 **Plenary Lecture**
Eosinophils regulate local immunity during *Trichinella spiralis* infection
Judith A. Appleton

13:55 - 14:20 **Plenary Lecture**
"Let's seal the deal and fight autoimmunity" says *Trichinella* to dendritic cells
Ljiljana Sofronic-Milosavljevic

14:20 - 14:35 Evaluation of different immunization protocols to induce protective immunity to *Trichinella spiralis* using TSL1 antigens in a murine experimental model
Guadalupe Ortega-Pierres

14:35 - 14:50 Effects of *Helicobacter pylori* neutrophil - activating protein on the protective role of IgE, eosinophils and on myositis in experimental trichinellosis
Fabrizio Bruschi

14:50 - 15:05 Exposure of pigs to encapsulated sylvatic isolates of *Trichinella* protects against a challenge infection with *T. spiralis*.
Dolores Hill

15:05 - 15:20 Vaccination against *Trichinella spiralis* in pigs : a high protection induced by a combination of recombinant proteins
Pascal Boireau

15:20 - 15:35 Some aspects of humoral and cellular immune responses in swine experimental *Trichinella spiralis* infection
Miruna OLTEAN

15:35 - 15:50 Effect on apoptosis associated protein expression of H7402 cells co-incubated with polypide protein of *Trichinella spiralis*
Xuelin Wang

15:50 - 16:05 Antigen-specific T cell immune response by co-immunization with the Ts87 DNA vaccine and recombinant Ts87
Yaping Yang

16:05 - 16:20 *In vitro* analysis of cellular activation of mouse mast cells stimulated by *Trichinella* antigens
Nicolas Versillé

16:20 - 16:40 POSTER EXHIBITION

16:40 - 18:00 ICT BUSINESS MEETING

5th August, Friday

16:30 - 17:00 **2nd ICT EXECUTIVE COMMITTEE MEETING**
Chair: Ray Gamble

17:00 - 17:45 **STUDENTS AWARD**
Chair: Christian Kapel

Trichinella spiralis infection in the large intestine follows a distinct profile compared to infection of the small intestine.
Lisa Blum

17:45 - 18:30 **HIGHLIGHTS AND CONCLUSION**
Ray Gamble, Mingyuan Liu

POSTER EXHIBITION SCHEDULE

2nd August, Tuesday

- Standardizing Science-Based Control Measures for *Trichinella* in Global Pork Trade
- Retrospective analysis of proficiency testing results of analysts in laboratories performing *Trichinella* digestion assay
- Towards a standardised surveillance for *Trichinella* in the European Union
- The *Trichinella* Reference method - interactive CD-ROM
- *Trichinella* detection: Identification and statistical evaluation of sources of error in the official reference method
- How safe is the meat inspection system using the artificial digestion of pooled samples: a scenario in a *Trichinella* non endemic region in Europe.
- Study on the suitability of inactivated *Trichinella spiralis* for proficiency samples
- Working team for trichinellosis - a systematic approach to prevention, control and eradication of trichinellosis in the Republic of Croatia
- Differences in the prevalence of *Trichinella* sp. in domesticated and wild boar influence the public-health consequences of insensitivity of diagnosis by trichinelloscopy: a census survey in an endemic region of Romania
- Computerization of PT management according to the ISO/IEC 17043:2010 standard
- *Trichinella*-situation in Belgium
- Physical and chemical factors and their influence on digestion of meat samples for *Trichinella* detection; the research made towards harmonization of veterinary law of Russia and the EU.
- The opportunistic nature of *Trichinella* - exploitation of new geographies and habitats
- Rat's defence against *T. nativa* is multifactorial
- Experimental infection with *Trichinella* T12 in domestic cats
- High prevalence and low level of *Trichinella* infection in domestic pigs of northern and eastern Henan, China
- *Trichinella* infection in fox (*Vulpes vulpes*) in Slovenia
- *Trichinella* infection in Mustelidae (Mammalia: Carnivora) from Romania: new host-parasite associations in a highly endemic country
- *Trichinella* infection in herbivores, do we wish to know more?
- Assessment of the presence of *Trichinella* spp. in rodents that live near pig farms in an endemic region of the province of Buenos Aires, Argentina
- *Trichinella pseudospiralis* in wild boars (*Sus scrofa*) of the Czech Republic
- Serological survey of trichinellosis in dogs in the French Mediterranean Island of Corsica

- Inflammatory phenotype of *Trichinella spiralis* nurse cell inferred from transcriptome analysis
- Molecular cloning and identification of a cystatin gene TsCystatin1 from *Trichinella spiralis*
- Transcriptome of small regulatory RNAs in the development of the zoonotic parasite *Trichinella spiralis*
- Protein changes of *Trichinella spiralis* infective larvae and intestinal epithelial cells after co-culture *in vitro*
- Is it possible to define the pattern of *Trichinella* sp. infection for human and pig sera by western blot?
- Protein changes of *Trichinella spiralis* muscle larvae *in vitro* induced by bovine bile
- Immunoscreeening c-DNA library of muscle larva of *Trichinella spiralis* and bioinformatic analysis of novel genes
- Epitopes scanning of newborn larvae stage-specific antigenic gene T668 in *Trichinella spiralis*

3rd August, Wednesday

- Current state and control of trichinellosis in endemic countries
- Current status of trichinellosis in Serbia
- Characteristics of the Epidemiological Process in the Development of Human Trichinellosis in Brasov County-Romania during 1983-2007
- An update on the *Trichinella britovi* focus on the island of Sardinia, Italy
- Expanded Zoogeographical territory of *Trichinella britovi* in Iran
- The EU perspective on risk management for trichinellosis
- A novel method for the detection of *Trichinella* in swine meat: *Trichin*-L
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- Evaluation of a novel bead-based assay for simultaneous determination of specific anti-body responses against *Trichinella spiralis* and *Toxoplasma gondii* in *porcine* serum
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- Establishment and initial Application of Real-Time PCR Detection Kit for *Trichinella* isolates in muscles
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- Characterization of DAF-21/HSP90, an antigen protein from parasitic nematode *Trichinella spiralis*

- Production and characterization of monoclonal antibodies against a serine protease from newborn stage of *Trichinella spiralis*
- Towards the first international pig reference serum with anti-*Trichinella* antibodies: isochronous studies on the reference swine serum candidates
- Digestion of individual meat samples for *Trichinella* detection as a substitute of compressor method.
- Development of a serological test to detect the humoral immune response against *Trichinella zimbabwensis* in Nile crocodiles (*Crocodylus niloticus*)
- Inspection of *Trichinella spiralis* pre-encapsulated larvae in muscle samples of experimentally infected mice
- Immunochromatographic strip for detection of anti-*Trichinella* antibodies in muscle juice of experimentally infected mice with low level infections
- Detection of anti-*Trichinella* antibodies in serum of experimentally infected swine by Immunochromatographic strip
- Application of liquid gene chip technique for detecting main food-borne parasites
- *Trichinella*: What's going on during nurse cell formation?
- Analysis of the effect of the new benzimidazole derivate GNV14, on *Trichinella spiralis* muscle larvae
- A new formulation of mebendazole in low-substituted hydroxypropylcellulose (L-HPC): improved efficacy against experimental trichinellosis
- TGF-beta and hedgehog signalling pathways in *Trichinella spiralis*
- Antigen genes display of *Trichinella pseudospiralis* at different development stages
- *Trichinella spiralis* and *Caenorhabditis elegans* display a parallel pattern of thymidylate synthase localization
- Freezing resistance of *Trichinella* muscle larvae in wild boars experimentally infected
- Serine proteinase inhibitor (Serpin)-A potential good diagnosis target for *Trichinella* late infection
- Potential roles of insulin-like growth factors in different pathology of infected muscle cells between *T. spiralis* and *T. pseudospiralis*
- Morphometric and molecular characterization of early intestinal stages of *Trichinella spiralis*.
- Rcd1 protein secreted from *Trichinella spiralis* down-regulates myogenin and MyoD proteins in C2C12 myoblasts, and inhibits binding activities of transcription factors NF-κB and AP-1.
- Carriage of *Trichinella* as a form of symbiotic relations
- Identification and Characterization of Deoxyribonuclease in Excretory/ Secretory Products of *Trichinella spiralis*
- Inhibition of Mammalian Muscle Differentiation by Excretory/ Secretory Products from *Trichinella spiralis*

- Characterization of a High Frequency Gene Encoding a Strongly Antigenic Cystatin-like Protein from *Trichinella spiralis* Muscle Larvae in the Earliest Intestinal Stage
- The effects of Nitazoxanide on *Trichinella spiralis in vitro* and *in vivo*

4th August, Thursday

- Sequencing the genome of *Trichinella spiralis*: learning from the past
- Shared and disparate patterns of developmentally regulated gene expression in two agents of human trichinellosis.
- Molecular identification of nematode larvae different from those of the *Trichinella* genus detected by muscle digestion
- *Trichinella* spp. infections in different host species of an endemic district of Serbia
- Cross-breeding between *Trichinella* T12 and the other encapsulated *Trichinella* species suggests its reproductive isolation
- Inferring the history of *Trichinella* diversification and dissemination from analyses of genetic variability: Recent achievements, future potential, and practical limitations
- Use of Nuclear Microsatellites in Genetic Variability Assessment of *Trichinella* Isolates
- Travel: a New Driver for Trichinellosis
- The Worldwide Occurrence and Impact of Human Trichinellosis, 1986-2009
- Clinical Manifestations in Human Trichinellosis - Retrospective Epidemiological Study
- Seroprevalence of *Trichinella* antibodies in blood donors in France
- Description of an outbreak of human Trichinellosis in an area of Argentina historically free of infection: Importance of surveillance studies
- Bear Meat related trichinellosis: an emerging zoonosis amongst French tourists in the Canadian Arctic
- A 10 Year Retrospective Epidemiologic Survey of the Diagnostic Errors and their Implications in Human Trichinellosis Development in Brasov County, Romania
- Eosinophils regulate local immunity during *Trichinella spiralis* infection
- "Evaluation of different immunization protocols to induce protective immunity to *Trichinella spiralis* using TSL1 antigens in a murine experimental model"
- Effects of *Helicobacter pylori* neutrophil-activating protein on the protective role of IgE, eosinophils and on myositis in experimental trichinellosis
- Exposure of pigs to encapsulated sylvatic isolates of *Trichinella* protects against a challenge infection with *T. spiralis*.
- "Let's seal the deal and fight autoimmunity" says *Trichinella* to dendrite cells.

- Vaccination against *Trichinella spiralis* in pigs: a high protection induced by a combination of recombinant proteins
- Some aspects of humoral and cellular immune responses in swine experimental *Trichinella spiralis* infection
- Effect on apoptosis associated protein expression of H7402 cells co-incubated with polypeptide protein of *Trichinella spiralis*
- Antigen-specific T cell immune response by co-immunization with the Ts87 DNA vaccine and recombinant Ts87
- *In vitro* analysis of cellular activation of mouse mast cells stimulated by *Trichinella* antigens
- Lung cells involved in the immune defense mechanism in *Trichinella spiralis* infection
- Invasion of mouse primary enterocytes *in vitro* by *Trichinella spiralis* infective larvae
- *Trichinella* as surgeons or influence of "Britov's vaccine" on the wounding process
- Immunological response against *Trichinella spiralis* infection in rats is dose dependent
- TNF-α induced myocardial apoptosis and affected lipid metabolism in *Trichinella spiralis*-infected mice
- Identification of an anti-tumor protein produced by *Trichinella spiralis* using a T7 phage display library
- Toll-like receptor activation by helminth or helminth products to alleviate inflammatory bowel disease
- The Protective Effect of the Effective antigen composition of *Trichinella spiralis* on Experimental Colitis in BALB/c Mice
- Differential immunological responses induced by infection with female adult worms or new born larvae of *Trichinella pseudospiralis*
- The effect on SP2/0 myeloma cell by *Trichinella spiralis* TS2 recombinant protein *in vivo*
- Growth Suppression Effects and Identification of The Differentially Expressed Genes by Infected with *Trichinella spiralis* Induced on SP2/0 Myeloma
- Anti-tumor Effect of Antibody against Associated Antigens between Hela Cell and *Trichinella spiralis*
- *Trichinella spiralis* infection in the large intestine follows a distinct profile compared to infection of the small intestine
- *Trichinella spiralis* infection during human pregnancy. A case report

ABSTRACTS

1 Standardizing Science-Based Control Measures for *Trichinella* in Global Pork Trade

H. Ray Gamble[a,*], Alvin A. Gajadhar[b]

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Keywords: *Trichinella spiralis,* pork trade, legislation, slaughter testing, quality assurance, International Commission on Trichinellosis

The *Trichinella* status of pigs has been a major factor in global pork trade for more than a century. For many countries, individual carcass testing and post slaughter processing by freezing or cooking have been the measures used to ensure public health and to meet export requirements. However, inconsistencies in *Trichinella* legislation between countries have contributed to barriers to trade. The ICT has made efforts to provide standards of best practices to the international regulatory community in its "recommendations" documents. New proposals for *Trichinella* control have been developed by some countries and are being further considered by international regulatory bodies such as the Office Internationale des Epizooties (OIE) and the Codex Alimentarius (Codex).These new measures focus on documentation of absence of risk and freedom from infection at the farm or region. The ICT has played a key role in recommending best practices for negligible risk pig management with respect to *Trichinella* and our work is cited by those now working on new documents in the OIE and Codex. International standardization is critical for ensuring equity among all countries in global pork trade. ICT members should play a key role in providing the knowledge to make science based recommendations regarding developing legislation. As an example of current ICT work in this area, a Quality Assurance (QA) Committee was established at the ICT Business meeting held during the joint WAAVP/AAVP/ICT conference in August, 2009 in Calgary, Canada. The purpose of this committee was to develop standardized procedures, recommendations, and other resources to facilitate quality assurance in the detection and control of *Trichinella*. A range of QA related needs were discussed and several tasks prioritized, including defining QA terms, digestion methods, proficiency testing, certification of *Trichinella* testing labs, and a training manual. This committee held a workshop in Maisons-Alfort, France in October, 2010 to work on the prioritized tasks, and 23 ICT members from 12 countries listed critical elements of various procedures, and a number of minimum criteria for standardization were developed. Work on the QA tasks is proceeding and will be advanced at the pre-ICT-13 QA meeting. A follow-up meeting is planned for November, 2011 in Rome to finalize work on the prioritized tasks and prepare documents for the ICT website and/or publications.

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2 Retrospective analysis of proficiency testing results of analysts in laboratories performing *Trichinella* digestion assay

Brad Scandrett[a,*], Lorry Forbes[a], Alvin Gajadhar[a], Pascal Boireau[b], Isabelle Vallée[b]

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Keywords: *Trichinella,* digestion assay, proficiency testing

In order to meet export, food safety, surveillance and ISO17025 quality assurance requirements, the Centre for Food-borne and Animal Parasitology (CFAP) of the Canadian Food Inspection Agency (CFIA) developed a system of digestion assay and proficiency testing for the detection of *Trichinella* larvae in pork and horsemeat. This also provided the basis for the development of a certification program for industry laboratories, with requisite analyst training and associated documentation for *Trichinella* testing to meet export requirements. Over the past decade we have accumulated and analysed proficiency sample performance data obtained for different numbers of larvae per sample, two different digestion assays, newly trained and experienced technicians, regulatory and industry laboratories, and from international proficiency sample exchanges. Results obtained indicate that the digestion assay proficiency sample system is fit for its intended purposes of training and monitoring analyst proficiency in the performance of *Trichinella* digestion assays in both regulatory and field applications.

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3 Towards a standardised surveillance for *Trichinella* in the European Union

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Keywords: *Trichinella;* Standardised monitoring; Risk-based surveillance; Pigs

Each year, more than 167 million pigs in the European Union (EU) are tested for *Trichinella* spp. under the current meat hygiene regulations. This imposes large economic costs on countries, yet the vast majority of these pigs test negative and the public health risk in many countries is therefore considered very low. This work reviewed the current *Trichinella* status across the EU as well as the national level of monitoring and reporting. It also reviewed which animal species were affected by *Trichinella* and in which species it should be surveyed. This information was used to design a cost-effective surveillance programme that enables a standardised monitoring approach within the EU. The proposed surveillance programme relies on identifying sub-populations of animals with a distinct risk. Low-risk pigs are finisher pigs that originate from so-called controlled housing. All other pigs are considered high-risk pigs. Controlled housing is identified by application of a specific list of management and husbandry practices. We suggest that member states (MS) be categorised into three classes based on the confidence that *Trichinella* can be considered absent, in the specified sub-population of pigs above a specified design prevalence which we set to 1 per million pigs. A simple and transparent method is proposed to estimate this confidence, based on the sensitivity of the surveillance

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system, taking into account the sensitivity of testing and the design prevalence. The probability of detecting a positive case, if present, must be high ($\geq$95 or $\geq$99%) to ensure that there is a low or negligible risk of transmission to humans through the food chain. In MS where the probability of a positive pig is demonstrated to be negligible, testing of fattening pigs from sub-populations consisting of pigs from controlled housing can be considered unnecessary. Furthermore, reduced testing of finishers from the sub-population consisting of pigs from non-controlled housing might even be considered, if conducted in conjunction with a proportionate sampling scheme and a risk-based wildlife surveillance programme where applicable. The proposed surveillance programme specifies the required number of samples to be taken and found negative. A MS with no data or positive findings will initially be allocated to class 1, in which all pigs should be tested. When a MS is able to demonstrate a 95% or 99% confidence that *Trichinella* is absent, the MS will be allocated to class 2 or 3, in which the testing requirement is lower than in class 1.

4 The *Trichinella* Reference method - interactive CD-ROM

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Keywords: *Trichinella* testing, Reference method EC 2075/2005, training, critical control points, food safety

This CD-ROM presents an introduction to the Reference method for the detection of *Trichinella* larvae in meat: the method for pooled sample digestion (according to the European Regulation EG/2075/2005). It was developed with the view to provide continuous quality improvement in the routine laboratory. It offers a step by step guide to the test process to everyone involved in the *Trichinella* analysis of meat. The procedure is split into several different chapters, so that one can quickly move through all the steps, or go directly to a specific section as required. Extra information and important points can be found by clicking on the FAQ button. There is also an 'Equipment & reagents' chapter, and a folder with Images. The CD-ROM is made for use in three languages: English, French and Dutch. The English version will be presented.

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5 *Trichinella* detection: Identification and statistical evaluation of sources of error in the official reference method

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Effective *Trichinella* diagnosis and control are essential for ensuring a high level of consumer protection. To assure the quality of the magnetic stirrer method (MSM) considered as reference method for *Trichinella* detection and to evaluate the competence of laboratories to detect and count *Trichinella* muscle larvae in pork samples, ring trials are conducted under the direction of the European National Reference Laboratories for *Trichinella*. According to literature, relevant sources of error in quantitative analyses occur at the following steps of the official procedure: sampling (improper samples, mix-up), sample preparation (inadequate grinding), digestion (unsuitable/expired chemicals, inadequate time/temperature parameters), sifting (sieve size), sedimentation (sedimentation time too short/long), and visualisation (erroneous diagnosis).

Experiencing ourselves one outlier when participating at the 2009 ring testing in Germany, we initiated a study to evaluate the reliability of MSM. First results presented here are based on (i) data of ring tests in Germany (2008, 2009, and 2010), (ii) in-lab performance in high repetition of the MSM using defined standards as well as (iii) recovering experiments, and (iv) statistical evaluation of these data. Quantitative data of the ring tests show that on average only about 60% of *Trichinella* larvae were detected (2008: 54%, 2009: 61% 2010: 59%) by laboratories with a large variability. The laboratories, which showed a comparably good performance (> 80% larvae recovery rate, no false negative results, no surplus larval count), frequently reported one sample of the four positive samples with an unexpected low larval count (loss of > 2 larvae). At our own laboratory, high numbers of repetitive analyses of standards (10 or 20 larvae) and re-analyses of residual fluids supported the suggestions that these "outliers" could be approximately described by a binomial distribution based on a laboratory-specific *Trichinella*-detection probability.

As our recovery experiments demonstrated, only a part of the total larval losses (16.5%) in the MSM within our lab (ca. 33%) could be explained by the already known critical steps. The major contribution, however, was due to so far unknown sources of error, which we detected on the level of sedimentation and visualisation. Considering the need to identify the critical control points according to the modern food safety concept of HACCP, our results indicate that a clear definition of analytical uncertainty including all sources of errors is required to guarantee this high claim of *Trichinella* detection methods.

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6 How safe is the meat inspection system using the artificial digestion of pooled samples: a scenario in a *Trichinella* non endemic region in Europe.

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In 2009, a 69 year old Dutch male patient was presented in a general district hospital showing loss of muscle power in the lower extremities and profound hypoalbuminemia and hypoproteinemia, following the day after admission with development of edema and serum albumin dropping, rhabdomyelosis and raised creatine kinase. The patient was transferred to a tertiary care hospital and required intensive care treatment. Most striking clinical findings were generalized edema. The patient gradually improved after supportive care with cefataxime, albumin and corticosteroids. By now the eosinophils were raised to 30% and the diagnosis trichinellosis was confirmed by serology. The Regional Health Services and the Food and Product Safety Authority were contacted to track the source of infection. A detailed food consumption list of the patient and his wife of the last 2 months before the onset of clinical symptoms was made, but no food source could be identified. The question raised if this patient could have obtained in the Netherlands, despite the absence of *Trichinella* in the Dutch swine population. Based on the transmission risk model of the lowest infection rate in rats, a scenario analysis was conducted from infected rats, to pigs to humans. In this scenario it was shown that given a test sensitivity using the artificial digestion method of 100% when 1 larva or more was present in 100 gram pooled tested pig meat or 40% when less than 1 larva was present, the digestion would fail 153 times in 10,000 simulations and consequently 35 humans per 10,000 inhabitants would develop trichinellosis when 100 gram of raw pork meat was consumed. Based on this scenario, it was shown that humans can obtain trichinellosis when only very few larva from wildlife enter the pig farm, being ingested by pigs and even when the animals all test negative, the meat might remain a risk for human consumption if eaten undercooked. In conclusion, the added value of testing indoor controlled housing raised pigs with the artificial digestion might raise questions given the sensitivity of the current test.

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7 Study on the suitability of inactivated *Trichinella spiralis* for proficiency samples

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Keywords: *Trichinella,* inactivated larvae, proficiency samples, magnetic stirrer method

Meat from food animals susceptible for *Trichinella* spp. must undergo an examination by the artificial digestion method. Personnel involved in the examination should be adequately trained and routinely participate in a quality control programme. For this purpose, ring trials are performed where *Trichinella* positive and negative samples must be identified correctly and in case of positive findings the number of larvae quantified. Usually, positive proficiency samples are prepared by spiking a certain number of live *Trichinella* larvae into meat samples processed from minced pork. Due to the use of infectious material, proficiency samples must be sent to the ring trial participants under special precautions. Further, after examination of the samples, the glassware and laboratory waste must be treated with hot water to efficiently kill the larvae. This also prevents the re-introduction of the larvae into the environment, which is particularly important in non-endemic regions. Therefore, this study aimed to evaluate the suitability of inactivated *Trichinella* larvae for proficiency samples. Muscle larvae (*T. spiralis,* ISS 003) were obtained by artificial digestion (magnetic stirrer method). The larvae were washed in water and one half was stored in 0.9% sodium chloride at 4°C over night. The second half was inactivated in 5% formalin at 4°C overnight. On the next day, 20 meat samples processed from negative minced pork (10g) were spiked each with 10 live and dead larvae, respectively. Meat samples with live or dead larvae were stored at 4°C for 1 week and 3 weeks, respectively. At the end of the storage period, each of the 10 meat samples was examined together with 90g negative pork by magnetic stirrer method according to Directive EC (No.) 2075/2005. Larval recovery rates for meat samples containing either 10 live or dead larvae and stored either 1 or 3 weeks were analysed by means of t-Test ($p \geq 0.05$). Larvae killed by 5% formalin did not change their coiled shape or internal structure in comparison with live larvae. However, the larval recovery rate for meat samples stored over 1 week and spiked with live larvae (98%) was significantly higher than for meat samples spiked with dead larvae (86%); $p=0.009$. Analogous results were obtained for the meat samples stored over 3 weeks (96% and 81%, respectively); $p=0.002$. Protocols for formalin inactivation were adapted to optimize the recovery rate for dead larvae and further results will be presented and discussed.

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8 Working team for trichinellosis - a systematic approach to prevention, control and eradication of trichinellosis in the Republic of Croatia

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Keywords: trichinellosis, prevention, control, eradication

At the end of the last century human trichinellosis was an important public health problem in the eastern parts of Croatia. However, the largest percentage of clinically diseased people was registered in Vukovar-Srijem county (up to 60% of all registered diseased people in Croatia), as well as 95% *Trichinella*-positive swine carcasses of all examined swine carcasses in Croatia, originated from Vukovar-Srijem county. Besides the health threat, trichinellosis implied notable economic expenses and endangered the traditional way of life and diet. In order to reduce all negative consequences of the disease there was a "Work team for trichinellosis" established - a group of scientists and experts from different fields of work who have helped and contributed a lot to minimize the threats of trichinellosis and maintain traditional processing and consumption of swine meat. The members, work methods and results of the Work team will be illustrated in this presentation.

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9 Differences in the prevalence of *Trichinella* sp. in domesticated and wild boar influence the public-health consequences of insensitivity of diagnosis by trichinelloscopy: a census survey in an endemic region of Romania

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Keywords: prevalence, *Trichinella* sp., pigs, wild-boar, trichinelloscopy, Romania

Trichinella represents one of the major foodborne parasites within the EU (Pozio et al., 1998), and is the principal zoonosis of Romania (Blaga et al., 2007). A retrospective analysis on human trichinellosis and animal *Trichinella* infection in Romania indicate a recent decrease in human cases from 3,649 in 1993 to 350 in2006, and for animal cases from 10,540 in 1993 to 674 in 2006, respectively. In spite of this declining trend, regional foci of veterinary and human cases persist, mainly due to poorly managed swine farms and a lack of veterinary inspections. In fact, no human cases have ever been reported from consumers of pork that has been subjected to veterinary inspection. The aim of the present work was to quantify the actual consequences, to public health risk, of performing diagnostic methods known to lack perfect sensitivity when applied in rural settings to either domesticated pigs derived from small household producers or to hunted wild boar. Our sample comprised tissues collected from all 1519 pigs and 11 wild-boars, submitted for veterinary inspection in the Transylvanian region of Romania (Cluj and Harghita Departments) from December 2009 through January 2010.These pigs did not derive from large swine farms (where artificial digestion is the required means of inspection) but rather from back-yard pigs raised, individually, on each of the 1519 farms. Trichinelloscopy continues to be practiced in such circumstances, and each specimen had been designated as negative by that method. We assumed that artificial digestion would provide a more sensitive means to diagnose infections in such animals, and (assuming perfect sensitivity with this alternative method) evaluated the

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predictive value negative of trichinelloscopy as applied in this circumstance. That is, we wished to know the probability that an animal was not infected, given a negative test by trichinelloscopy. To do so, artificial digestion was performed on pools of 10 samples (10 g/sample). No infections were identified among the 1519 backyard pigs, but infection was detected in 1 of the 11 wild-boars (3.6 larvae per gram).Thus, all husbanded pigs that had tested negative by trichinelloscopy were also found by artificial digestion to harbor fewer than 0.1 larvae/gram (predictive value negative = 100%).However, the predictive value negative for the test, when applied to wild boar, was less (90.9%) owing to the occurrence of infection in one of 11 animals that had tested negative by trichinelloscopy. We conclude that trichinelloscopy may contribute to safeguarding public health under certain, challenging field conditions (where it is logistically difficult to perform more sensitive testing by means of artificial digestion). However, where prevalence rates are significantly greater, the insensitivity of trichinelloscopy engenders greater public health risk. Where the risks of false negative test are unacceptable, greater diagnostic sensitivity is required.

10 Computerization of PT management according to the ISO/IEC 17043:2010 standard

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Keywords: Proficiency Tests (PTs), computerization, ISO/IEC 17043:2010 standard, European Union Reference Laboratory for Parasites (EURLP*)*

Scientifically sound and uniform testing is an element of fundamental importance for appropriate disease diagnosis, therefore one of the tasks of the European Union Reference Laboratory for Parasites (EURLP) is to organize Proficiency Tests (PTs) for the National Reference Laboratories for parasites of the European member states, aimed to verify and improve the performance of such laboratories in conducting specific diagnostic tests. In the last 5 years, the EURLP organized yearly PTs on pooled sample digestion methods for the detection of *Trichinella* larvae in fresh meat, according to the European Commission Regulation (EC) 2075/2005. Besides concurrent schemes, also sequential PTs were organized for the Italian public and private laboratories performing these methods. In order to improve the management of these activities, the EURLP developed a computing system on the web portal. The system was designed to comply with the ISO/IEC 17043:2010 requirements in terms of design and operations of PT schemes, data analysis and evaluation of results, reports, communications with participants and confidentiality. The system was built by developing software made up by two applications: one intranet type to be utilized by the ISS personnel and one web type to be used by laboratories. The intranet software allows us to manage samples to be sent to participating laboratories and to verify their data entry. The web software was developed on ASP.NET platform with VB Script programming language to get and elaborate test sample results. Authorized users can login through an authentication system placed into an infrastructure based on Active Directory, a Certificate Authority issuing the cryptography certification. Users can fill in a form with laboratory data, request to participate to the scheduled PTs schemes and/or ask for samples for training and audit purposes, or for method validation. As soon as laboratories entry test results, a printable report is generated, containing, for each sample, the test results and the z-score, together with an evaluation of the overall laboratory performance and EURLP recommendations based on laboratory outcome. All data are recorded in a Microsoft SQL Server database cluster protected by a three stages recording system, and automatically backed up.

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11 *Trichinella*-situation in Belgium

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Keywords: *Trichinella*, Belgium, food safety, monitoring

In Belgium, *Trichinella* testing is mandatory in pigs, wild boar and horses according to EU legislation. Mandatory testing is performed in routine laboratories and furthermore the National Reference Laboratory for Parasites is dedicated to do the confirmation of suspected larvae and quality control. The results of digestion and serological tests in food safety testing and several surveys and monitoring programs over the past years will be reported, as well as the recently obtained 'negligible risk status' for finisher pigs and the monitoring of wildlife. No *Trichinella* larvae have been detected in pork or horsemeat since the imposed testing. The prevalence of *Trichinella* spp. in wildlife in Belgium is very low. Each year about 10,000 wild boars are routinely inspected for *Trichinella*. There were only two positive findings with low intensities of *T. britovi* in 2004 and unspecified larvae in 2007. In animals destined for food consumption, testing for *Trichinella* with the digestion method continues, but there is derogation for finisher pigs kept under specified circumstances.

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12 Physical and chemical factors and their influence on digestion of meat samples for *Trichinella* detection; the research made towards harmonization of veterinary law of Russia and the EU.

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The differences of veterinary regulations to detect *Trichinella* larvae in meat samples by digestion in artificial gastric juice gained more significance due to recent attempts for unification of legalized methods. Prohibition of compressorium method usage according to the EU Regulation #2075/2005 has also driven much interest to the magnetic stirrer method and its equivalents among the laboratories performing routine slaughter meat tests. We have carried out an investigation of the equivalence between the magnetic stirrer method of pooled samples according to #2075/2005 and current Russian method of the same analysis. The influence of physical-chemical and physical parameters of digestion process on efficacy of *Trichinella* larvae release have been tested and allow to make the following conclusions: The recipe of artificial gastric juice, temperature and digestion\sedimentation phase duration prescribed in magnetic stirrer method are more effective than used in Russia. In the respect of sensitivity for dead larvae release the European method concedes the method used in Russia. Recommendations of samples collection from diaphragm pillar; without fascia and fat, described in EU Directive are difficult to perform in massive routine tests – best way would be to double the weight of meat taken and to loosen the requirements to the volume of not digested residue.

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13 The opportunistic nature of *Trichinella* - exploitation of new geographies and habitats

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Keywords: *Trichinella*, distribution, habitat, carnivorism, mammals, birds, reptiles

For more than one-hundred years *Trichinella spiralis* (former *Trichina spiralis*) was considered a zoonotic parasite of the domestic habitat involving pigs, synanthropic rats and humans. In the last 70 years, there were increasing evidences that the biomass of nematodes of the genus *Trichinella* is greater in wild than in domestic animals and the domestic foci of *T. spiralis* represent only an epiphenomenon of the natural cycle of these helminths. Omnivores and carnivores animals (mammals, birds and some reptiles) mainly those with cannibalistic and scavenger behaviour play the main role of reservoir of the 12 *Trichinella* taxa recognised so far. Even if *Trichinella* spp. do not develop in invertebrates, muscle larvae ingested by arthropods can be spread in the environment by these paratenic hosts. The distribution areas of the *Trichinella* spp. hosts can help to identify the environmental suitability where the different *Trichinella* taxa can be detected. Both the survival of larvae in decaying muscles of their hosts which is favoured by high humidity and low temperatures and the human behaviour in the domestic and wild habitats, play a role in the transmission patterns of these nematodes. *Trichinella spiralis* from its probable area of origin, i.e. South-Eastern China, first spread to Europe probably by the wild boar and then it invaded passively the American Continent, New Zealand, and Egypt during the European colonization. *Trichinella pseudospiralis* spread in at least three continents by birds and then, at least in Europe, the wild boar seems to play the most important role of reservoir. The ancestral species of the extant 'sylvatic' species colonised by carnivore mammals Africa, North and South America, Asia, and Europe originating a new taxon in each region: *T. nativa* in the Arctic, *T. britovi* in Eurasia and North-Western Africa, *T. murrelli* in temperate regions of North America, *T. nelsoni* in Eastern Africa, *Trichinella* T6 in the arctic region of North America, *Trichinella* T8 in Southern Western Africa, *Trichinella* T9 in Japan, and *Trichinella* T12 n.sp. in Southern America. The non-encapsulated species *T. papuae* and *T. zimbabwensis* invaded both the aquatic and terrestrial environments by the infection of crocodiles, monitor lizards and carnivore and omnivore mammals. Even if *Trichinella* taxa develop in different hosts species circulating in different geographical regions, there is a common denominator among the hosts: their scavenger behavior.

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14 Rat's defence against *T. nativa* is multifactorial

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Keywords: *T. native, T. spiralis*, defence, innate immunity

Trichinella nativa is the major sylvatic species in boreal environment. Rats are considered to be important vectors from sylvatic cycle to domestic. However, they are regarded as selective hosts for *T. nativa*: the infection level in muscles remain low. Our aim was to identify in which phase of the life cycle selective responses take place in a rat. *T. spiralis* and *T. nativa; in vitro* reproduction was assessed, larval burdens after p.o. and i.v. infections compared, and serological responses analyzed. Adult *Trichinella* females were collected from rat intestines on day six after *per os*, and cultured *in vitro*. In one day, *T. spiralis* female produced three times more new born larvae than *T. nativa* (70 NBL *vs.* 23 NBL, P<0.001). Isolated NBL were injected into rat's tail vein. The infection intensity in the muscles (larvae per gram, lpg) was examined after five weeks of the infection (p.o. infection with 2000 muscle stage larvae and i.v. with average 4700 NBL per rat) by artificial digestion of the euthanized and eviscerated rats. Both p.o. and i.v. *T. spiralis* infected animals produced more muscle larvae than *T. nativa;* p. o. 1300 lpg *vs.* 31 lpg and i.v. 6.6 lpg vs 0.52 lpg; respectively. The average infection percentage (total number of muscle larvae in rat / total dose X 100) with i.v. *T. spiralis* infected rats (n=6) was 24% and with *T. nativa* infected (n=9) 2.0% (p<0.01). Per os *T. spiralis* infection percentage was 10 000% *vs. T. nativa* 300% (p<0.001). Thus, with enteral reproduction, *T. spiralis* produced 33 times more muscle larvae than *T. nativa,* whereas in the parenteral phase, after i.v. administration, *T. spiralis* survived to muscle stage larvae 12 times more often. *Per os* infected rats showed seroconversion with in-house ELISA in two weeks, and both p.o. and i.v. infected at the termination of the experiment. In both infection routes *T. nativa* responses were statistically similar to *T. spiralis* responses. The reproduction capacity of the two *Trichinella* species isolated from rat is different; this certainly influences on the infection level detected later in the muscles. However, the difference between the infection levels of *T. spiralis* and *T. nativa* remains also in i.v. administration when the enteral phase, along with the reproduction have been bypassed; the defense against *T. nativa* is not solely enteral in rats.

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15 Experimental infection with *Trichinella* T12 in domestic cats

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Trichinella spiralis has been documented in different wild animals, puma, armadillos, rats and wild boars, in Argentina. In 2008 a new taxon, named *Trichinella* T12, was identified by molecular analysis in a cougar (*Puma concolor*) from the Patagonia. The aim of the present work was to study the relationship of infectivity and pathology of *Trichinella* T12 between the puma and the domestic cats and the possible risks that it could represent for transmission among these animals. Two cats (A and B) were orally infected with larvae of *Trichinella* T12 and one cat was used as control. Cats A and B were inoculated with 3300 larvae and 1850 larvae respectively. During the 54 days post-infection (dpi), daily examination, body temperature, cardiac and respiration rates were measured and then were euthanized. At 7 day intervals thereafter, cats were bled by cephalic venipuncture and blood samples were collected. Haematological studies included: hematocrit (%), hemoglobin (g/dl), and white cell, neutrophil, lymphocyte and eosinophil counts. Blood biochemistry included: bun, creatinina, AST, ALT, CK, LDH and ALP. Serum samples from both animals were tested by ELISA for the presence of *Trichinella* antibodies before and throughout the experimental period. At necropsy, some organs (liver, spleen, brain, cerebellum and kidney), nails and muscles samples were obtained for histopathology studies and artificial digestion. The muscles studied were diaphragm, massetters, cutaneous, temporal, intercostals, lumbar, tongue, muscles of legs, hands, neck and tail. Clinical signs as anorexia, diarrhoea, vomiting, shaggy hair, decay and muscle pain were observed in both cats. The values of eosinophils were increased in cats A and B. No antibodies were detected before inoculation and the highest serological response was observed after 40 dpi until the end of the experience in cats A and B. The histopathology showed larvae in several muscles without degenerative reaction. In the organs analyzed were not observed larvae neither lesions. *Trichinella* larvae were recovered from all the muscles analyzed. The cat A showed a maximum of 246 lpg in temporal muscle and a minimum of 80 in the tongue, while the cat B showed a maximum of 65 lpg in muscles of the leg and a minimum of 10 larvae in tail muscles. It is the first record of experimental infection in cats with *Trichinella* T12.

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16 High prevalence and low level of *Trichinella* infection in domestic pigs of northern and eastern Henan, China

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From 1984 to 2003, 16 outbreaks of human trichinellosis, consisting of 521 cases, were reported in southern, western and central parts of Henan province, central China. Pork is the predominant source of outbreaks of human trichinellosis in Henan. Out of 16 outbreaks, 12 (751%) were caused by eating raw or undercooked pork. However, no trichinellosis was recorded in Anyang and Shanqiu located in northern and eastern Henan, where they were historically designated as trichinellosis-free area. The aim of the present work was to determine the prevalence of swine *Trichinella* infection in these above two areas. A total of 475 diaphragm muscle samples (363 indoor-raised pigs and 112 industrialized raised pigs) were collected in abattoirs of Anyang and Shanqiu during 2010 to 2011. Muscle samples were examined by direct microscopic examination and artificial digestion method. No *Trichinella* larvae were detected by direct microscopic examination. *Trichinella* larvae were found in 18 of the 475 pigs (3.79%) by digestion method. The prevalence of swine *Trichinella* infection was 4.46% (9/202) with larval loads of mean 0.48 (0.1-1.58) muscle larvae per gram (lpg) in Anyang and 3.3% (9/273) in Shanqiu with larval loads of mean 0.27 (0.33-1.41) lpg, respectively. All 18 positive samples were from indoor-raised pigs. However, all 112 pigs from industrialized pig farms were negative for *Trichinella* infection. Out of 18 positive samples, 4 (22.22%) samples had the larval loads ≥1 lpg, the minimum level of infection considered to be of public health concern. The larvae were identified by multiplex PCR as *Trichinella spiralis*. Our study confirms the existence of porcine trichinellosis in northern and eastern Henan regarded as *Trichinella*-free areas, and indicates that failure to report cases of trichinellosis based on inadequate surveillance can result in incorrect prevalence classification of an area. The results are useful for evaluating the risk of infection for humans, and indicate that the indoor-raised pigs seem to play an important role in the maintenance of the domestic cycle of *T. spiralis* in northern and eastern Henan.

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17 *Trichinella* infection in fox (*Vulpes vulpes*) in Slovenia

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Different species of *Trichinella* infest birds, reptiles and various mammals, including humans. The specialty of *Trichinella* is that they first infest the intestine and then the skeletal muscles of a host. The epidemiology of trichinellosis is very complex due to different species of *Trichinella*, a large number of potential hosts and the existence of different life cycles. Sylvatic cycle is independent of human as a host and is related to wildlife population, in which *Trichinella* are mostly transmitted among carnivores via prey and carrion. *T. spiralis* is mostly transmitted in the synanthropic cycle in which the causative agents circulate within the population of pigs. Pigs get infested with insufficiently heat-treated garbage and animal waste products, by ingesting pig carcasses and probably also by biting tails and ears. Wild animals, e.g. foxes and rats, may also represent a source of infection for pigs. It has been demonstrated that elimination of these animals from the pig environment contributes to the interruption of the life cycle. Skeletal muscle samples of the lower forelimb and diaphragm of 1288 foxes were investigated for the presence of larvae. In case of insufficient quantity of the primary sample, adequate quantities were assured by additionally/substitutionally investigating the muscles of the upper forelimb, tongue and masseter. The prevalence of *Trichinella* spp. larvae in foxes in Slovenia, calculated for the hunting season 2006/2007 on the basis of investigation of skeletal muscles, was 0.534%.

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18 *Trichinella* infection in Mustelidae (Mammalia: Carnivora) from Romania: new host-parasite associations in a highly endemic country

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According to a survey of the International Commission on Trichinellosis, Romania has the most cases of human trichinellosis in the world, with both the domestic and the sylvatic cycles present. Wild animals represent the most important source of infection for domestic pigs and through these, to humans. The only two species of *Trichinella* identified so far in Romania are *Trichinella spiralis* and *Trichinella britovi* but none of the previous few studies in mustelids provided data on molecular identification of species in these hosts. The aim of this study was to identify by molecular tools the *Trichinella* species distribution in mustelids from Romania. Between 2009 and 2011, 19 specimens belonging to the family Mustelidae have been examined: *Martes foina* (n=3), *M. martes* (n=2), *Meles meles* (n=4), *Mustela erminea* (n=3), *M. lutreola* (n=3), *M. nivalis* (n=1), *M. putorius* (n=2), and *Vormela peregusna* (n=1). After artificial digestion, PCR was used for identification of *Trichinella* species. The infection was found in *M. erminea* (3/3), *M. lutreola* (1/3) and *M. foina* (1/2). PCR analyses revealed the presence of two *Trichinella* species. *Trichinella britovi* was found in two *M. erminea* and in *M. foina* and *Trichinella spiralis* was found in one *M. erminea* and *M. lutreola*. No mixed infections were detected. This is the first report of genus *Trichinella* in *M. erminea* and *M. foina* from Romania and the first molecular proof of *Trichinella spiralis* in Mustelidae from Europe. The finding of *T. spiralis* highlights the possible implication of this group of carnivores in the flow of this parasite from the domestic to the sylvatic cycle.

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19 *Trichinella* infection in herbivores, do we wish to know more?

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During the last few decades, *Trichinella* species have been found to infect a broad spectrum of vertebrates excluding fishes and amphibians. The only natural way of infection known is still by swallowing muscle larvae in tissues of an infected host. So, the infection is highly associated with predating and scavenging. The omnivore, pig, has traditionally been regarded as the most important infection source to humans. However, one of the most important origins of human infection lately has been found to be horse meat. Equines are typical herbivores and them getting sporadically infected shows that they may not be strict vegans. The same is true with many other herbivorous animals as well. In an old study, *Trichinella* muscle larvae were found in snowshoe hares in Alaska. Hares are claimed to be cannibalistic under availability of dead conspecifics, which behavior will aid them to survive under harsh conditions. Also the arctic reindeer do eat rodents, and lemmings have been found in Norwegian reindeer rumen. Even though carnivorism may be rare in herbivores, equines are not only exceptions. Experimental infections of ruminants led to production of muscle larvae both in sheep, goats, cattle and reindeer. Even though the reproductive capacity has often (not always) been low, the same is true with horses. Experimentally, *Trichinella nativa* has failed to establish in ruminants, or only caused a very low grade infection, unlike *T. spiralis* and *T. pseudospiralis*. Both the exposure and susceptibility to *Trichinella* infection may be present more often than imagined for many herbivorous animals. Therefore, maybe herbivores play a concealed role in the epidemiology of sylvatic *Trichinella* infection in the Arctic and sub-Arctic, perhaps elsewhere also. However, very seldom have human *Trichinella* infect ions been traced back to consumption of meat of other herbivores than horses. This might partly be due to ignoring them as self-evidently non-hosts for *Trichinella* spp, leading to underestimating their role. To better understand sylvatic *Trichinella* epidemiology and transmission to humans we should know more about infection in herbivores.

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20 Assessment of the presence of *Trichinella* spp. in rodents that live near pig farms in an endemic region of the province of Buenos Aires, Argentina

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Trichinellosis is a wide spread food borne zoonosis caused by species of the genus *Trichinella*. Until present *T. spiralis* is the only species usually found in porcine and non- porcine animals from Argentina. Nevertheless, Krivokapich et. al. isolated in 2008 a new taxon of *Trichinella*(*T.* T12). *T. spiralis* can be transmitted and maintained in both a domestic and sylvatic cycle whereby rats, among others contribute to the spread of *T. spiralis* from domestic to sylvatic animals and vice versa. The aim of this research was to determine rat species living pig farms , the presence of infected rats in areas where porcine trichinellosis had been present or not, to examine *Trichinella* species presented in rat carcasses and to analyze its relationship with this parasite whether brown rats acts as a reservoir of the disease or as a an accidental host. In this study we analized the presence of *Trichinella* infection in rodents which inhabit pig farms from General La Madrid, Buenos Aires, Argentina. For this purpose 9 pig farms with different levels of sanitation and with, or without, *T. spiralis* infected pigs and a garbage dump were assessed between spring 2008 and winter 2009. Pig farms were classified with 2 criteria: sanitation level and presence of *Trichinella* infection (positive/negative). Once animals were captured they were identified and euthanized in order to take samples from different muscles: diaphragm, tongue, masseter, intercostal muscles, and the limbs. A total of 150 rodents were captured. All the species belonged to the genus *Rattus* species *norvegicus*. The presence of *Trichinella* spp was tested by artificial digestion of each muscle sample. Samples were analyzed by artificial digestion resulting in negative outcomes (no presence of *Trichinella* in any individual). Studies in wild animals such as wild boars, opossums, wild carnivores (mustelids, procyonidae, etc.) carried out in different parts of the world, including Argentina contribute to reconsider the existence of a natural reservoir other than the brown rat The absence of positive rats in both negative and positive pig farms reinforce the hypothesis of the rat as a vector or an accidental host rather than a reservoir of the disease.

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21 *Trichinella pseudospiralis* in wild boars (*Sus scrofa*) of the Czech Republic

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Keywords: *Trichinella pseudospiralis*, wild boar, Czech Republic

Trichinella pseudospiralis is a non-encapsulated species infecting both mammals and birds. In the Czech Republic, more than 120,000 wild boars (*Sus scrofa*) are hunted per year and most of them are tested for *Trichinella* by artificial digestion (e.g. 121,185 were hunted and 96,232 were examined in 2009). In December 2010 and January 2011, *Trichinella* sp. larvae were detected in three wild boars hunted in the eastern part of the Czech Republic. The larvae were identified as *Trichinella pseudospiralis* by multiplex-PCR. All *T. pseudospiralis*-positive wild boars had similar weight (around 35 kg) and were shot at the same baited site by the same hunter. A common origin of the infection in the three wild boars has been hypothesized: 1) the meat used as bait for attracting the wild boars was the source of *T. pseudospiralis* infections; or 2) the three wild boars belonged to the same wild boar herd which had fed on the same carcass of an infected wild animal. These findings support the tendency of a more frequent detection of the non-encapsulated species *T. pseudospiralis* in Europe, which probably is related to the increased number of tested wild boars and to the use of the artificial digestion instead of the less sensible trichinoscopy to detect these zoonotic parasites in meat samples. The importance of the hunting practices for spreading *Trichinella* sp. infection among wild animals is also stressed.

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22 Serological survey of trichinellosis in dogs in the French Mediterranean Island of Corsica

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Keywords: *Trichinella britovi*, dogs, Corsica, serology, epidemiology

In February 2010, *Trichinella britovi* was detected for the second time in the French Mediterranean Island of Corsica. The parasite was found in outdoor pigs, in a remote valley in the centre of the island, at less than 10 km from the previous positive herd found in 2004. This first case brought to light the emergence of the parasite on the French island, considered until now as *Trichinella* free. First investigation in the surrounding wildlife revealed one contaminated fox. Consecutively to these findings involving both domestic and wildlife, an epidemiological survey was conducted in the entire island on 1881 wild boars and 74 foxes from 2006 to 2008. No *Trichinella* larva was recovered in wild boar or in foxes but an apparent serological prevalence in wild boars of 2.01%, suggested that Corsican wildlife was currently exposed to *Trichinella* (1). For this second case of pig trichinellosis, a serological survey was conducted on breeders and hunters' dogs living in the remote valley. An in-house E/S ELISA was set up using positive dogs' serum provided by the EU-RL Parasites and 444 negative serum samples from dogs collected in the veterinary school of Maisons Alfort. Then, 297 serum samples collected on dogs living in the valley and 68 serum samples from dogs living in the neighboring valley were tested. The apparent serological prevalence of *Trichinella* infections in dogs was 3.5% (95% CI: 1.88% - 5.92%), among which one dog living in the neighboring valley is highly positive. All incriminated dogs are used for hunting and are older than 1 year. This serological survey confirms the presence of *Trichinella* in Corsica. Re-emergence of *Trichinella* in this remote valley can occur at anytime and the veterinary inspection of pigs is fundamental. Effectively, majority of pigs are traditionally used to be slaughtered in farm, without any veterinary inspection since the length of the valley ant its isolated location made difficult the access to a slaughterhouse and to sanitary service. Since a new slaughterhouse has opened in November 2009, the number of controlled pigs increased from 600 to 2000.

(1) Richomme *et al.,* Vet Parasitol. 2010; 172(1-2):150-4.

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23 Inflammatory phenotype of *Trichinella spiralis* nurse cell inferred from transcriptome analysis

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Muscle larvae of *Trichinella* encapsulating species reside in a modified portion of infected myofiber, called a nurse cell. Our studies of signaling pathways operating in *T. spiralis* nurse cell, analyzed based on expression microarray data with the use of Ingenuity Pathways Analysis (IPA) software, allowed to identify nurse cell growth arrest as senescence-associated G1-like type. Upregulation of genes encoding major histocompatibility complex (MHC) class II receptors, proteasome subunits, cathepsins, prostaglandin E synthase, complement components, interleukin (IL) 1 α and β, IL1 receptors, IL11 and tumor necrosis factor (TNF) α, indicated acquisition of immunological functions by nurse cell. Upregulated expression of MHC class II receptors as well as numerous proteases pointed to nurse cell capability of antigen presentation. Expression of IL1, IL11 and TNFα indicated a proinflammatory role of that cell. Currently, immunologically-relevant functions of *T. spiralis* nurse cell were further pursued. Upregulation of genes encoding master molecules of endocytic pathways, *i.e.* clathrin A and B, as well as caveolin 1, corresponds with the process of particle internalization performed by antigen presenting cells. Upregulated expression of Toll-like receptors indicate capability of nurse cell to recognize molecular patterns evoking immune response. Apart from IL1, IL11 and TNFα, nurse cell is found to express also other proinflammatory cytokines, including interferon γ and IL6. This remains in accord with proinflammatory role of senescence-associated secretory phenotype (SASP) factors, as ascribed to SASP of various cellular senescent systems. Nurse cell was also found to display upregulation of several components of NADPH oxidase complex, known to be responsible for superoxide generation on macrophage or phagosomal membrane. It thus seems to be capable of eliciting direct immune response, even though superoxide generated on the plasma membrane is also known to participate in growth factor or cytokine signal transduction. Expression of specific molecules, including transmembrane receptors, required for execution of immune functions, may result in recognition of nurse cell by host immune system as self and thus assure its existence throughout the host life-span.

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24 Molecular cloning and identification of a cystatin gene TsCystatin1 from *Trichinella spiralis*

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A cystatin cDNA sequence (TsCystatin1) of *Trichinella spiralis* was cloned using *in Silicon* cloning from GenBank database and RT-PCR. The results indicated that the cDNA sequence contained an open reading frame (ORF) of 666 nucleotides and the deduced polypeptide consisted of 221 amino acids with the theoretical molecular weight of 24.3 key and bioelectric point of 4.44. A signal peptide sequence of TsCystatin1 located between amino acids 1 and 25. The conserved domain QVVAG of cystatin, 3 N-glycosylation sites and 2 disulfide bonds were also identified. Structural domain analysis showed that the protein contained a cystatin-like domain belonging to the cystatin family and showing high similarity to those of other nematodes. Specific primers derived from TsCystatin1 cDNA sequence was used to amplify *T. spiralis* genome DNA and two extrons and one intron of 75 bp were identified. RT-PCR and real-time RT PCR indicated this gene was contiguously expressed at adult, newborn larval and muscle larvae stages. The TsCystatin1 cDNA was cloned into pGEX-4T-1 vector and expressed in *E. coli* system. Antiserum against the recombinant protein was prepared and a specific band was detected in adult and muscle larvae soluble antigen. The recombinant TsCystatin1 has also been demonstrated to possess inhibitory activity against cathepsins.

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25 Transcriptome of small regulatory RNAs in the development of the zoonotic parasite *Trichinella spiralis*

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Keywords: miRNA, endo-siRNAs, *Trichinella spiralis*, transcriptome

Small non-coding RNAs such as microRNAs (miRNA) and interfering RNAs (siRNA) execute post transcription regulation by translational repression or mRNA degradation. Currently, a large number of miRNAs have been identified in diverse species. In *T. spiralis*, the profile of small non-coding RNAs and their function remain poorly understood. The transcriptional profiles of miRNA and siRNA in three developmental stages of the ancient parasite *T. spiralis* were first investigated and compared by a high-throughput RNA sequencing technique, indicating unique sequences (5,443,641). Of these, 21 conserved miRNAs related to 13 metazoan miRNA family and 213 novel miRNAs unique to *T. spiralis* were identified. Some of these miRNAs exhibited stage-specific expression. Expression of these miRNAs was confirmed in three stages of the life cycle by qRT-PCR and Northern blot analysis. In addition, endogenous siRNAs (endo-siRNAs) were found mainly derived from natural antisense transcripts (NAT) and transposable elements (TE) in the parasite. Evidence for the presence of miRNAs and endo-siRNAs in *T. spiralis* has been identified and defined. MiRNAs accounted for the major proportion in the small regulatory RNA population of *T. spiralis*, while endogenous siRNA was much less. The finding of stage-specific expression patterns of the miRNAs in different developmental stages of *T. spiralis* suggested that they may play important roles in parasite development. The data provide a basis for further understanding of the molecular regulation and functional evolution of miRNAs in parasitic nematodes.

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26 Protein changes of *Trichinella spiralis* infective larvae and intestinal epithelial cells after co-culture *in vitro*

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The purpose of the present study was to investigate the protein changes of *Trichinella spiralis* infective larvae and intestinal epithelial cells after co-culture *in vitro* and to screen the proteins related with invasion. Muscle larvae were incubated in raw bovine bile with 1:20 dilution in PBS and the activated larvae were inoculated into culture of HCT-8 cells to incubate for 18h. The larval invasion into cells and development were observed by an inverted microscope after being incubated at 37°C under 5% CO_2 for different times. The proteins of muscle larvae and cells were extracted and then analyzed by SDS-PAGE and Western blot. The results showed that larvae invaded cell monolayer and shed their cuticle after culture for 2h and 18h, respectively. On Western blot, after cultured with HCT-8 cells, additional seven protein bands (123, 77, 58, 36, 30, 28, 20kDa) of larvae were recognized by sera of the infected mice, but three protein bands (97, 51, 23kDa) of larvae were not recognized by the above sera, compared with the larvae cultured in the medium without HCT-8 cells. The HCT-8 cells cultured with larvae had additional four bands (115, 61, 35, 24kDa) which could be recognized by sera of the infected mice, compared with normal HCT-8 cells. Our results showed that the protein components of infective larvae and HCT-8 cells changed after co-culture *in vitro*, suggesting that additional seven proteins recognized by sera of the infected mice may be the invasion-related proteins secreted by the larvae during the invasion, while the three proteins not recognized by the above sera may be related with the protein decomposition by proteases released by the invaded cells. After co-culture *in vitro* the, components of cell proteins also changed, additional four proteins recognized by sera of infected mice may be related with invasion of intestinal epithelial cells by infective larvae, these proteins might mediate or facilitate entry into the cells.

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27 Is it possible to define the pattern of *Trichinella* sp. infection for human and pig sera by western blot?

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Keywords: *Trichinella* sp. Infection, human, pig, serology, western blot, diagnosis

ELISA has a great value as a diagnostic tool for human infections and for monitoring programs aimed at verifying the *Trichinella*-free status in pigs from high containment level farms. This test shows a high sensitivity, but it may also result in a low specificity due to false positive reactions. Consequently for a diagnostic purpose, a confirmatory test such as the western blotting (wb) is required to confirm the infection in both human and pig ELISA-positive sera. The aim of the present study was to identify the most frequently *Trichinella* specific antigens recognized by both human and pig sera on excretory/secretory antigens (ESA) by wb, in order to define the pattern of recognition for positive sera. To this end, 143 human sera (51 from people with a confirmed diagnosis of trichinellosis; and 92 from people without *Trichinella* sp. infection, but with other infections or autoimmune diseases, which cross-reacted by ELISA, CRS) were tested. Moreover, 114 ELISA-positive pig sera were tested: 1) 17 from experimentally *Trichinella* sp. infected pigs; 2) 62 from naturally *Trichinella* sp. infected pigs; and 3) 35 ELISA cross-reacting sera (CRS) from *Trichinella* free pigs. All sera from persons with a confirmed diagnosis of trichinellosis reacted with the three bands of 53-70 kDa (first band from 53kDa to 55kDa, second band from 59kDa to 62kDa and third band from 67kDa to 70kDa). Other proteins were recognized by the 51 sera with different frequency. The human CRS recognized different proteins on the *Trichinella* ESA by wb. Sera from experimentally infected pigs reacted with three bands of 48-72 kDa (first band from 48kDa to 55kDa, second band from 59kDa to 63kDa and third band from 64kD to 72kDa); whereas, not all the sera from naturally infected pigs recognised these three bands. None of the 35 ELISA CRS from *Trichinella*-free pigs recognised the three bands.

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28 Protein changes of *Trichinella spiralis* muscle larvae *in vitro* induced by bovine bile

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The purpose of this work was to investigate the protein changes of *Trichinella spiralis* muscle larvae induced by bovine bile *in vitro*. The larvae were activated by 5% raw bovine bile diluted in saline at 37°C in 5% CO_2 for 2h, and then crude antigens of the activated larvae were prepared. The larvae were also activated by 5% bovine bile in serum-free RPMI-1640 medium for 18h at 37°C in 5% CO_2, and their excretory-secretory (ES) antigens were collected. The crude and ES antigens were analyzed by SDS-PAGE and Western blot. On SDS-PAGE, additional two protein bands (56, and 21 kDa) of crude antigens of the activated larvae were observed, and one protein band (17 kDa) was disappeared, compared to crude antigens of no-activated larvae. On Western blot, after the larvae were activated by bile, additional three (133, 125, and 26 kDa) and four (125, 116, 80, and 29 kDa) protein bands of crude antigens were recognized by sera of the mice infected with *T. spiralis* and immunized with ES antigens, respectively; however, two (106 and 25 kDa) and three (76, 58, and 16 kDa) protein bands were not recognized by the above two kind of sera, compared to the crude antigens of no-activated larvae. When the ES antigens containing 5% bovine bile were analyzed by SDS-PAGE, additional two protein bands (39, and 31 kDa) were observed, compared to individual ES antigens and bile. On Western blot, Additional four (136, 39, 38, and 36 kDa) and seven (136, 120, 100, 39, 36, 34, and 31 kDa) protein bands of ES antigens containing 5% bovine bile were recognized by sera of the infected mice and immune sera, respectively; but two (67, and 20 kDa) and ten (132, 112, 33, 32, 26, 23, 21, 19, 16, and 15 kDa) protein bands were not recognized by the above two kind of sera respectively, compared to individual ES antigens and bile. The results showed that after the larvae were activated by bile, their protein components changed, suggesting that additional one protein (125 kDa) in crude antigens and three proteins (136, 39, and 36 kDa) in ES antigens may be the proteins secreted by the activated larvae, these proteins may be related with invasion of intestinal epithelial cells by *T. spiralis* infective larvae.

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29 Immunoscreeening c-DNA library of muscle larva of *Trichinella spiralis* and bioinformatic analysis of novel genes

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Keywords: *Trichinella spiralis*, Sp2/0 myeloma cell, immunoscreen, cDNA library; bioinformatics; related antigen

To obtain novel vaccine candidate antigens of anti-tumor vaccine, cDNA library of muscle larva of *Trichinella spiralis* was screened by using sera of Balb/C mouse immunized by Sp2/0 myeloma cell. The fragment was amplified using T3 and T7 primers and phage as templates. Recombinant phage was excised *in vivo* using the ExAssist helper phage with SOLR strain. Total length of gene was incised by restriction enzymes EcoR I and Hind III, and was amplified by PCR after insertion fragment of positive clone was acquired by 5'-RACE. The positive clones were also sequenced and the data were analysed through the internet Nucleotide BLAST software of NCBI and Expert Protein Analysis system of ELM and DNAStar software. One positive clone was obtained after three rounds of immunoscreening to 1.9×10^6 pfu/mL cDNA library. The sequencing results showed that this insert was 569 bp in length and contained a single open reading frame (ORF) and it was predicted to encode 136 amino acids after the cDNA fragment was extended by 5'-RACE technology and cloned into pMD-T18 vector. This gene was designated TS2 and was gene of *T. spiralis* by BLAST searches with the GenBank database. ELM analysis showed that TS2 protein has four N-Arg dibasic convertase (nardilysine) cleavage site one peptide C-terminal amidation site, one glycosaminoglycan attachment site, DNAStar software analysis in antigenic index showed that TS2 protein has six epitope sites. Conclusion one gene predictably encoding related protein between Sp2/0 myeloma cells and *T. spiralis* is obtained from immunoscreening of muscle larvae of *T. spiralis* cDNA library bysera of Balb/C mouse immunized by Sp2/0 myeloma cell.

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30 Current state and control of trichinellosis in endemic countries

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It is clear that *Trichinella* is not an endangered species. It is still present in muscle cells of many pigs, wild animals and some humans despite all measures that have been implemented for more than a century. This is especially true for the various endemic countries in Europe, Asia and South America. In the majority of southeastern European countries, cases of trichinellosis among the human and animal populations were described in the late 19th or early 20th centuries. A high prevalence of trichinellosis in domestic animals and humans has been reported in Bulgaria, Serbia, Montenegro, Romania, Croatia and Bosnia and Herzegovina. It has to be stressed that during the post-war period, a dramatic increase in the number of positive pigs was a consequence of breaches of control measures in previously known endemic regions of Croatia. The following prevalence rates clearly demonstrate the high risk for humans practicing the traditional food habit of eating cured pork products. In the period between 1997 and 1999, 600 240 slaughtered pigs were tested for *Trichinella*, and 0.16% of them were found to be positive. The loss due to the disease was estimated almost 10 431 800 Euros per year. Almost two decades later (2011), the prevalence rate showed a significant decrease when only 53 animals were found infected. This decrease was a result of 15 years of government-funded intensive monitoring and control activities. The greatest success within the eradication program was achieved through continuous rodent control at all sites where infected pigs were detected, prompt disposal of infected swine carcasses, and compensation to the owners for disposed pigs. Social and economic factors responsible for the emergence of trichinellosis in southeast Europe as other parts in the world will be discussed.

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31 Current status of trichinellosis in Serbia

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Keywords: *Trichinella*, trichinellosis, Serbia

In Serbia, trichinellosis has been a human health and animal husbandry problem for almost a century. It is possible to recognize its presence in almost all parts of the country today. Areas of highest swine infection represent nearly one third of the country. The increase of infection rate among swine was noticed after the period 1980-1990 (0.009-0.02% incidence in swine, 100-200 cases of human trichinellosis/ year) when political, social changes and wars took place. The period 1990-1999 was characterized by re-emergence of swine trichinellosis in Serbia: increase of incidence up to 0.17% in 1999 among swine, accompanied by a significantly higher number of human cases (more than 500 on average per year). In this century, the application of measures based on newly issued guidelines (Ministry of Agriculture, Forestry and Water Management, R. Serbia, Veterinary Directorate) resulted in some improvements of meat control methods. The rate of swine infection gradually decreased from 2001 to 2010 (below 0.05%) which resulted in significant reduction in number of infected people (fewer than 200 cases per year). Prevention and eradication of human trichinellosis is a common task of veterinary and health services. In order to help *accomplish this task,* INEP as National Reference Laboratory for Trichinellosis (NRLT) is continuously engaged in surveillance and control of this disease, as well as in promotion *of* public awareness regarding safe meat and meat products consumption.

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32 Characteristics of the Epidemiological Process in the Development of Human Trichinellosis in Brasov County-Romania during 1983-2007

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Keywords: direct factors, indirect factors, epidemiological process, correlations

Trichinellosis involves considerable economic losses in diagnosis and also constitutes a public health problem via high levels of morbidity and its chronic nature, socially and professionally debilitating for the former patient. Objectives and Methods The analysis of the results from epidemiological surveillance of human trichinellosis outbreaks, identifying direct and indirect factors of epidemiological process and their share in maintaining the endemics-epidemics in Brasov county. Retrospective epidemiological study conducted over a period of 25 years (1983-2007) on a group of 3,345 consumers. Data were derived from epidemiological outbreak investigations and of the observation sheets provided by the Public Health Authority, Infectious Diseases Hospital, Family Physicians offices from Brasov county. There were a total of 246 outbreaks involving 3,345 consumers, of which 2,179 were patients; between 1987 and 1997, the number of outbreaks reached alarming proportions (202), with 2,503 consumers and 1,660 patients; between 2000 and 2007 their number decreased significantly: 18 outbreaks with 378 consumers, of which 231 were patients, demonstrating the efficiency of National Program of Surveillance and Control of Trichinellosis in humans and animals, implemented after 2000. There were 177 outbreaks in urban and 69 outbreaks in the rural areas. Urban areas represented 83% of all cases compared to 17% in rural areas; the urbanization process of the disease is apparently, the source of infection being the contaminated pig increased in their rural households. The seasonal distribution of diseases reports a significant proportion of summer-autumn months (15%), raising the idea of parasite movement on other new ways of transmitting undetected by us. Multi-annual trend of the sources of infection indicate an obvious decrease in the frequency of infected pigs detected in slaughterhouses, while the trend of infected pigs in the households is ascending. There is a very small percentage of illnesses due to game meat (0.9%). Current practice has shown the large number of outbreaks recorded in the years when the pork trichinelloscopic examination was performed after pig slaughter or at the first signs of the disease. The implementation of the National Program of Surveillance and Control of Trichinellosis in humans and animals lowered the incidence of this disease in humans.

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33 An update on the *Trichinella britovi* focus on the island of Sardinia, Italy

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Keywords: *Trichinella britovi*, Italy, human outbreak, pig, epidemiology

Until to 2005, the Island of Sardinia (Italy) of the Mediterranean basin had been considered free of *Trichinella* sp. in domestic and wild animals and in humans. In 2005, two human outbreaks involving 19 people and in 2007 a single case of trichinellosis, occurred by consumption of pork from 3 free ranging pigs infected by *Trichinella britovi* in the village of Orgosolo. The epidemiological investigation carried out in the Orgosolo municipality on 681 free-ranging pigs revealed the presence of four infected pigs (0.6%), but no infection was detected in wild boars or foxes hunted in the island. A passive introduction of the parasite from the neighbouring island of Corsica (France) was suspected. In January 2011, a new human outbreak of trichinellosis (5 hospitalised people) and a single hospitalised person occurred in the same village of Orgosolo by consumption of pork from pigs free ranging in the same municipality. The epidemiological investigation carried out on 350 free-ranging pigs, revealed the presence of 8 *T. britovi* infected animals (2.8%). The average age of all the 16 infected pigs detected in 2005-2006 and in 2011 was 5.5 (range 1-10) and most of them (13) were sows. The average number of larvae per gram in the diaphragm of the 16 pigs was 62.9 (range 0.1-348). The free-ranging pigs originating from municipalities neighbouring Orgosolo tested always negative. The outbreak of *T. britovi* infection in humans and in pigs of the Orgosolo municipality four years after the first one opens two epidemiological questions: 1. is *T. britovi* circulating in the area and could the outbreaks represent only an epiphenomenon?; or 2. could the 2005-2007 and the 2011 outbreaks be related to two independent passive introductions of *T. britovi* from outside Sardinia? The discovery of *T. britovi* in Corsica in 2004 and its circulation among free ranging pigs and wild animals up to the recent years seem to be related. There is a great exchange of people and goods from Corsica to Sardinia, including the importation of wood. It has been assumed that some free-ranging pigs have been illegally introduced from Corsica to Sardinia, hiding the pigs under wood in the lorry or in the boot of cars.

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34 Towards a surveillance for *Trichinella* in Slovenia

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In Slovenia, routine trichinoscopic examination in the muscle tissue of the domestic pigs in the slaughterhouses started in 1928. In 1929, *Trichinella* spp. were detected in 2 pigs. Both pigs were bought in Serbia. Between the years 1952 and 1956 were slaughtered 131,473 pigs. In this period, one case *Trichinella* was found. This was the last documented case of trichinellosis in a domestic Slovenian pig. The last outbreaks of human trichinellosis in Slovenia were detected in 1983 and 1989. In both cases, pigs came from Serbia and were slaughtered at home. During the period 2000 to 2010 in Slovenia, slaughterhouses slaughtered 4,549,000 pigs. The population of domestic pigs substantially decreased in the last decade. In 2000, 510,648 pigs were slaughtered in slaughterhouses; in 2010, only 289,303 pigs were slaughtered. All slaughtered pigs in slaughterhouses were examined with the artificial digestion method and were negative. The European Union regulation enables acquiring of different *Trichinella* status. Maintaining such status requires a surveillance program on region or breeding. Part of the surveillance is possible with serological methods. The suitability of different commercial ELISAs ware checked for our conditions. In December of 2010, we collected 803 blood samples and diaphragm pillars in two large Slovenian slaughterhouses. The serum samples were tested by three commercial ELISA tests and they were negative in all three tests. The examination of diaphragms was preformed in accordance with Commission Regulation (EC) No. 2075/2005 with the magnetic stirrer method for pooled sample digestion and all samples were also negative.

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35 Expanded Zoogeographical territory of *Trichinella britovi* in Iran

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PCR diagnosis enables progress in understanding the geographic distribution of species of *Trichinella.* In Iran, *Trichinella* has been known in carnivores in several parts of the country. Since pork consumption is prohibited in Islamic beliefs, human infection is not a major public health concern among observant Muslims. However, the infection risk should not be underestimated for Christian minorities, who may consume wild boar meat on occasion. Two such human cases were documented in the last three decades. Isolates from the Caspian and Khuzestan regions in the southwestern portion of the country previously sent to Boew in Alma-Ata in former USSR, identified these as *T. spiralis* and *T. nelsoni*, respectively. The first PCR based speciation on this genus in Iran was that of a *Pantera pardus saxicolor* in northwestern the country that was found naturally infected with *T. britovi*. Expectation of *T. britovi* existence, regarding to its synonym species *T. nelsoni*, in mentioned region, which have been thought to be merely limited in southwestern parts of Iran, was exciting. Aiming to expand our information on *Trichinella* species throughout the country, sampling from road killed carnivores of three provinces in the south and north were commenced. Based on the latest findings using 5S and ITS genes sequencing, obtained isolates from Khuzestan province in southwest have identified as *T. britovi*.

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36 The EU perspective on risk management for trichinellosis

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Keywords: *Trichinella*, meat inspection, legislation, European Union

The White Paper on Food Safety introduced a radical new approach for food safety and consumer protection and was approved by the European Commission in 2000. The main objective was the implementation of a new legislation covering all aspects of food products from "farm to fork", the development of national control systems for food safety and improvement of the communication between consumers and stakeholders. In this context the so called "EU hygiene package" consisting of several EU Regulations which focus on food hygiene and food safety measures came into force at the beginning of 2006. With regard to the control of trichinellosis Regulation (EC) No. 854/2004 lays down specific rules for the organisation of official meat inspection system and Regulation (EC) No. 2075/2005 focuses on specific rules for official controls for *Trichinella* in meat. Thus, all domestic and wild animals intended for consumption and susceptible for *Trichinella* infection (e.g. pigs, horses and wild boars) must be examined under supervision of the competent authority. Recently, many efforts were made to improve the quality assurance system by means of training campaigns and ring trials for laboratories involved in *Trichinella* meat inspection. Furthermore, these laboratories must undergo accreditation in accordance with ISO/IEC 17025 what means a huge challenge from the financial and organisational point of view. Considering the specific epidemiological situation in EU Member States, a risk based approach (i.e. the derogation from a systematic *Trichinella* inspection) can be applied for fattening pigs which come from farms or regions where the risk of an infection is negligible. Regulation (EC) No. 2075/2005 lays down specific obligations for the food business operator and the competent authority regarding management issues accompanied by a monitoring programme in order to control the maintenance of the status of the certified farm or region. Besides the need for better harmonized monitoring programmes good solutions are desirable for an efficient logistic slaughter system for pigs which must or must not undergo *Trichinella* inspection. For an assessment of the current epidemiological status all Member States must provide data on human trichinellosis and *Trichinella* findings in domestic and wild animals in accordance with Directive 2003/99/EC. Results published annually by the European Food Safety Authority show that the epidemiological situation differs widely between various countries and regions. Some countries/regions are free from *Trichinella*, whereas in other areas the domestic and/or sylvatic *Trichinella* cycles exist. However, the critical evaluation of data also reveals the need for further improvement of the reporting system to better verify the epidemiological situation in the future.

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37 A novel method for the detection of *Trichinella* in swine meat: *Trichin*-L

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Keywords: Parasitic zoonoses, *Trichinella*, pepsin digestion, filtration, nylon membrane, monoclonal antibodies, latex detection

Trichinellosis is a zoonotic disease caused by nematodes of the genus *Trichinella*. Domestic pigs are the primary source of infection. In Human, trichinellosis are mainly caused by ingestion of poorly cooked meat infected with *Trichinella*. In Europe, Commission Regulation (EC) N° 2075/2005 of December 5, 2005 is laying down the rules on official controls for *Trichinella* in meat. The Digestion method is today the only EU approved method for *Trichinella* inspection. It involves a direct detection of the parasite by microscopic observation following digestion of pooled meat with pepsin. Bio-Rad recently developed a new method for the detection of *Trichinella* antigens in swine meat. This new method, called *Trichin*-L, is based on the same assay principle as the digestion method but with a simplified assay protocol. It also involves preliminary digestion of pooled meat with Pepsin. Then, sample sedimentation steps required in the EC approved digestion method are replaced by a single filtration on a nylon membrane. Finally, latex beads coated with specific anti-*Trichinella* monoclonal antibodies allow the detection of even low quantities of *Trichinella* antigens. The latex detection assay format does not require highly trained technicians and greatly reduce the risk of missing *Trichinella* larvae in digested pooled meat. The test allows detecting up to one larva in a 100g-meat pool and recognizes all usually encountered *Trichinella* species. Recent comparative evaluations have shown that the *Trichin*-L assay protocol is approximately 50 minutes shorter than the official digestion method. The same evaluations also showed that the technicians in charge of testing spent 10 minutes less for each sample pool. The new method was successfully evaluated at the end of 2010 by the Community Reference Laboratory for *Trichinella* (ISS Roma, Italy) and four other National Reference laboratories in Europe. The new method is now pending EU approval and commercialization of the product should start in 2011.

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38 New artificial digestion assay-An alternative to the current pepsin digestion

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The artificial digestion method is the procedure currently recommended to detect *Trichinella* larvae in meat. It is based on an artificial digestion with pepsin to release muscle larvae from meat and the subsequent identification by microscopy. According to the EC Regulation 2075/2005 each individual pig carcase must be analysed for the presence of *Trichinella* larvae. The PrioCHECK® *Trichinella* AAD was developed to be used as an alternative artificial digestion method to the current pepsin digestion for the detection of *Trichinella* larvae in meat. The new method is based on a serin-endopetidase of the enzyme group subtilisine. In a first step, meat from experimentally infected pigs was digested to demonstrate the functionality of the method. Larvae could be detected from all tested samples. Ten pieces of 10 g pig meat spiked with 3 up to 24 *Trichinella* larvae, six samples containing *T. spiralis* larave, two samples containing 3 and 8 *T. pseudospiralis* larvae and two samples containing 10 and 20 *T. britovi* larvae were analysed. Additionally, two samples from wild boars containing 3 and 7 *T. spiralis* larvae, two horse samples containing 4 and 9 *T. spiralis* larvae, respectively, and one horse sample containing 40 *T. britovi* larvae were analysed. A high recovery rate was obtained for all samples and was comparable to the conventional artificial digestion using pepsin. In a second step proficiency samples were prepared and digested with the new artificial digestion method and compared to the conventional pepsin artificial digestion. A total of 20 samples of 10 g containing 10 larvae were prepared and analysed. All samples were correctly classified and the average recovery rate was 87.5%, whereas the average recovery rate with the pepsin digestion was 97%. With the PrioCHECK® *Trichinella* AAD, larvae are inactivated during the digestion, but they are morphologically intact and strain typing with PCR can be done without limitations. These results show that the PrioCHECK® *Trichinella* AAD is a valuable alternative to the traditional method, which allows a standardized and quality assured *Trichinella* inspection. Further evaluations and validations will be done to prove the suitability of the new enzyme used for the artificial digestion of meat samples.

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39 Multispectral direct detection of *Trichinella* larvae on nylon filters

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Globally there is a need to cost-effectively document that pigs raised under such conditions are free of *Trichinella*, but a rational approach is complicated by the fact that the occurrence of both pig and human trichinellosis differ markedly between countries globally and even within regions. Although several new methods for *Trichinella* surveillance are being developed and risk based herd certification is a potential alternative to classical meat inspection, *Trichinella* testing by digestion assays are still widespread and considered the gold standard. A new vision-based method and instrumentation has been developed for the direct detection of *Trichinella* larvae recovered from a range of digestion processes used in classic meat inspection. The process allows for subsequent molecular analysis and thereby a thorough risk analysis of potential findings. The instrument consists of a table-top scanner unit and a PC which runs the application software. The user prepares the sample on a dedicated sample holder (35um net), inserts the sample into the instrument and activates the software. The instrument then scans the entire surface of the net, capturing several multispectral images, which are analysed to form a combined analysis of the entire sample. The result of the analysis is displayed on the screen after a couple of minutes. The core of the instrument is a multispectral vision unit which is specially constructed to acquire the imagery. It has a variety of light sources with different wavelengths and a number of specially selected filters for combined reflectance and fluorescence measurements. The illumination, filtering, multispectral image acquisition of the sample, and analysis is set up in a recipe using a standard PC. The images captured are processed using dedicated analysis algorithms. The analysis reports an image where each pixel is labelled a *Trichinella* or not. *Trichinella spiralis* larvae were propagated in mice and released by artificial HCl-pepsin digestion. Known numbers were deposited on nylon filters. Sub-sampling form dilutions yielded a good correlation between manual and automated detection, but not completely identical, as sub-sampling from a dilution is not 100% accurate. Subsequently, the number of *Trichinella* on the tested nets was verified manually by microscopy. The number of pixels detected as *Trichinella* by the machine was in close correlation to the number of *Trichinella* confirmed by microscopy. A few samples with less coiled *Trichinella* produced a higher number of pixels per larvae as they cover a larger area, which may influence the precise

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quantification of larvae. At higher larvae number, a few samples produced more pixels than anticipated, but such "false positive" observations do not constitute a hazard to the end user and may be suppressed in later versions of the analysis software. More importantly, no false negative tests were observed. There is a close correlation between the manually verified number of *Trichinella* larvae recovered on a net and the number of pixels detected as "positive" by the instrument. The instrument is able to detect single larvae even in presence of muscle fibres. De-coiled larvae results in larger number of pixels, but over-estimation will not compromise consumer safety. No false negatives were found. Automation of the direct inspection methods may offer a cost effective method to certify meat free of *Trichinella*.

40 International standardization of *Trichinella* detection methods

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Keywords: standardization, *Trichinella* detection methods, ISO, CEN, European Union Reference Laboratory for Parasites (EURLP)

Harmonization and standardization of diagnostic methods is one of the tasks of the European Union Reference Laboratory for Parasites (EURLP) transmitted by food, designed by the DG SANCO of the European Commission in 2006. Therefore, the EURLP is carrying out some actions aimed at the standardization of analytical methods for the detection of parasites in food. Standardization is achieved through consensus agreements between national delegations representing all economic stakeholders, suppliers, users, government regulators and other interest groups. They agree on specifications and criteria to be applied consistently in testing and analysis, in the classification of materials, in the manufacture and supply of products, in terminology and in the provision of services. ISO, the network of the national standards institutes of 157 countries, and CEN, the European Committee for Standardization, on the basis of the Vienna agreement, published in June 1991, collaborate in order to increase transparency of work ongoing in CEN to ISO members, avoid duplication of work, increase the speed of elaboration, availability and maintenance of standards through a need to establish consensus only once. Most of Standards and other approved documents have been drawn up in Technical Committees. The ISO Technical Committee 34, Food products, Sub Committee 9-Microbiology (ISO/TC34/SC9), and CEN Technical Committee 275, Food analysis, horizontal methods, Working Group 6-Microbial contamination (CEN/TC275/WG6), collaborate in order to develop international standards of methods for the detection of microbial contaminants in food. In 2007, during a parallel meeting of the two committees held in Cairo, Egypt, a resolution (n. 149) was approved by the CEN committee members who agreed to launch work on a new topic by creating a new Technical Advisory Group (TAG), TAG 7-Parasites, giving priority to the work on *Trichinella*. In 2008 CEN/TC275/WG6 members requested the DG SANCO of the European Commission to send to the CEN/TC275/WG6 Secretariat a formal letter of agreement to standardize both the artificial digestion method for the detection of *Trichinella* in meat and serological methods for detection of antibodies to *Trichinella* in swine serum. At present, during the last CEN/TC275/WG6 meeting held in Bournemouth, UK, in June 2011, all members agreed to continue the TAG7 work on *Trichinella* at ISO level to speed up the standardization process.

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41 Validation of a latex agglutination test for the detection of *Trichinella* infections in pigs

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Keywords: latex agglutination, *Trichinella* detection, pig, artificial digestion.

An antigen detection kit (*Trichin*-L), based on latex agglutination test, developed by the Bio-Rad company has been validated at five European laboratories. According to the EURL for Parasites Guidelines approved by EU member state, the validation protocol included specificity, sensitivity, robustness and reproducibility assessments. Specificity was evaluated by testing parasites belonging to the *Anisakis*, *Ascaris*, *Toxocara*, *Toxoplasma, Trichinella* and *Trichuris* genera. As a control, 140 pork samples negative for *Trichinella* were used. For the sensitivity, 10 pork samples spiked with 1, 3, 6 or 15 *Trichinella* larvae were tested in each laboratory. To evaluate the robustness of the test, the solubilised antigens were maintained at room temperature and tested at different times (1, 4, 8, 12, 24, 48, 72 h). For the reproducibility, each laboratory tested 40 minced pork samples of 100 g each (30 spiked with *Trichinella spiralis* larvae and 10 negative). The use of the larval homogenate obtained using the *Trichin*-L kit as a template for the parasite identification at the species level by a multiplex PCR, was also evaluated. The results showed: 1) a high specificity, since only samples spiked with all the eight *Trichinella* species larvae tested positive; 2) a high sensitivity, because almost all samples spiked with only one larva tested positive; 3) the solubilised antigens maintained its stability and reactivity up to 3 days, suggesting a good robustness of the test; 4) the test reproducibility was high, because similar results were obtained in the five laboratories; and 5) later, the larvae were successfully identified at the species level after DNA concentration from the homogenate obtained using the *Trichin*-L kit. The evaluation of the *Trichin*-L method also allowed for the identification of changes in the test protocol that could impact assay performance. In particular, sample testing without preliminary sufficient meat chopping, and poor rinsing of the vessels after washing with high concentrations of detergents could impact the specificity of the method. In conclusion, the *Trichin*-L Antigen Test Kit meets the requirements for the accurate detection of *Trichinella* larvae in pork samples.

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42 Evaluation of a novel bead-based assay for simultaneous determination of specific antibody responses against *Trichinella spiralis* and *Toxoplasma gondii* in *porcine* serum

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Keywords: *Trichinella spiralis*, *Toxoplasma gondii*, bead-based assay, pigs, serology

A novel, bead-based flow cytometric assay was developed for simultaneous determination of antibody responses against *T. spiralis* and *T. gondii* in pig serum. This high throughput screening assay could be an alternative for well-known indirect tests like ELISA. One of the advantages of a bead-based assay over ELISA is the possibility to determine simultaneously multiple specific antibody responses per single sample run facilitated by coupling a series of antigens to identifiable bead-levels. Furthermore, inclusion of a non-coupled bead-level in the same run facilitates the determination of and correction for non-specific binding. The performance of this novel bead-based assay was compared to one commercially available *T. spiralis* ELISA, an in-house *T. gondii* ELISA and two commercial available *T. gondii* ELISA's. For this purpose, sera from *T. spiralis* and *T. gondii* experimentally infected pigs were used. With the infection status as gold standard, the area under the curve, Youden Index, sensitivity, specificity, negative and positive predictive values were determined through receiver operator curve analysis. Marginal homogeneity and inter-rater agreement between bead-based assay and ELISA's were evaluated using McNemar's Test and Cohen's kappa, respectively. Results indicate that the areas under the curve of the bead-based assay were 0.885 and 0.908 for *T. spiralis* and *T. gondii*, respectively, while that of the *T. spiralis* ELISA was 0.879 and that for the *T. gondii* ELISA's ranged between 0.837 and 0.930. The bead-based *T. spiralis* assay had a sensitivity of 68% and specificity of 100% while the ELISA scored 72% and 95%, respectively. Bead-based *T. gondii* assay had a sensitivity of 85% and specificity of 96%, while the ELISA's ranged between 64-84% and 93-99%, respectively. Results of the McNemar's Test showed that there was homogeneity in positive test proportions between the *T. gondii* bead-based test and one of the commercially available *T. gondii* ELISA's. Moreover, the comparison of this test combination, and the comparison between *T. spiralis* bead-based assay and respective ELISA showed an excellent inter-rater agreement. In conclusion, the new bead-based test, which detects *T. spiralis* and *T. gondii* antibodies simultaneously, can replace other indirect tests while performing equally well or better.

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43 Detection of *Trichinella spiralis* circulating antigens in serum of experimentally infected mice by an IgY-mAb sandwich ELISA

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In the present study, a sandwich ELISA based on IgY (egg yolk immunoglobulin) and monoclonal antibody (mAb) against excretory-secretory (ES) antigens of *Trichinella spiralis* muscle larvae was developed for detection of circulating antigens (CAg) in sere of mice infected with *T. spiralis*. The IgY-mAb sandwich ELISA involved the use of chicken antibody IgY as a capture antibody and mouse mAb 35B9 as a detecting antibody. This method was able to detect as little as 1 ng/ml of ES antigens added to normal mouse serum. A group of sixteen mice was orally inoculated with 500 *T. spiralis* muscle larvae per animal. The serum samples from the experimentally infected mice were taken during 1-49 days post-infection (dpi). The CAg was detectable as early as 3 dpi in the sera of infected mice. The level of CAg reached a peak at 13 dpi, and then declined gradually. Another peak of CAg was observed at 24 dpi. The anti-*Trichinella* antibodies was first detected in 33.3% of the infected mice at 3 wpi, and reached a peak positive rate of 100% at 5 wpi. Moreover, the infected mice were treated with abendazole at 5 weeks post-infection and the serum CAg levels increased significantly during 2-8 days post-treatment and then declined rapidly during 8-12 days post-treatment. By 40 days after treatment, the CAg levels decreased to the undetected level, but the anti-*Trichinella* antibodies were still detected in 100% of the infected mice. The novel assay appears to be sensitive for detection of antigenemia of *T. spiralis* and valuable to the early diagnosis and evaluation of efficacy of chemotherapy in trichinellosis.

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44 Establishment and initial Application of Real-Time PCR Detection Kit for *Trichinella* isolates in muscles

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Keywords: *Trichinella* isolates, detect, Real-Time PCR

Specificity primers and TaqMan MGB probe were designed and the target fragments of five *Trichinella* isolates were compared and analyzed. Standard preparation of 10^2 to 10^7 copies/μl was produced through dilution. All five *Trichinella* isolates were amplified with high specificity. The correlation coefficient was 0.9984, and even 132 copies could be detected after 40 cycles. Genome DNA of a single *Trichinella spiralis* was extracted and diluted, even 0.00001 single polypide could be detected by Real-Time PCR. The 20 reactions using the same sample were simultaneously detected; coefficient variation of these Ct values was 1.11%. The same batch of standard preparation was detected after 30 days; there was no significant deviation in the twice detection through hypothesis test. It indicated favourable repetitiveness of Real-Time PCR method. Analogical samples and practical samples were detected by Real-Time PCR Detection Kit for *Trichinella* isolates as semi-works production in this study, the former was mouse muscle infected with *Trichinella spiralis* and the latter was dog′s diaphragm for quarantine. Real-Time PCR could efficiently detect mouse muscle when larva got into muscle fiber, 2 weeks after infection, all analogical samples could be 100% detected regardless of high infection dose or low infection dose. Comparing with test under microscope and artificial digestion, Real-Time PCR has many features such as fast, accurate, high-flux, high sensitivity and specificity. Practical samples were also detected using the four methods, 8 weakly positive samples were detected from 63 samples by Real-Time PCR, and other 3 methods′ results were negative. It hinted that Real-Time PCR was more sensitive and adapted to requirement of quarantine.

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45 Excretory/secretory biomolecules from *Trichinella spiralis*/*T. britovi* L1 larvae as potential diagnostic markers

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Keywords: *Trichinella spiralis, Trichinella britovi*, excretory/secretory proteins, recombinant proteins, diagnosis

Trichinellosis is a worldwide zoonosis. Humans can be infected by consumption raw or inadequately cooked meat containing *Trichinella* larvae. Under the European Commission Directive (EC) No. 207502005 the magnetic stirrer method for pooled-sample digestion is recommended as a reliable method for routine detection of *Trichinella* larvae in meat. Serological tests can be used for monitoring, surveillance and certification purposes. Up to now, *Trichinella* larvae excretory/secretory proteins (ESP) are used as "complex" antigens and the unspecified mixture of ESP is one of the factors limiting the specificity of serological test. In our experiments, proteomics approach was used to analyse ESP from *T. spiralis* and *T. britovi*L1 larvae. The 2-DE of ESP proteins followed by MALDI-TOF/TOF-MS were used to analyse the protein spots and *Trichinella* EST databases were used for interpretation of recorded data. The specific primers for three protein/antigen representatives were designed, appropriate genes were cloned and their recombinant forms expressed using yeast (*Pichia pastoris*) and bacterial (*Escherichia coli*) systems. The recombinant antigens are currently being tested for their immunodiagnostic potential.

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46 Characterization of DAF-21/HSP90, an antigen protein from parasitic nematode *Trichinella spiralis*

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Keywords: *Trichinella spiralis,* Tsdaf-21/hsp90, phylogenetic analysis, expressing, western blot, immunolocalization

Trichinella is an important animal parasitic nematode in the world. As a pathogen, every year millions of people were under the health threat. *Trichinella* infects a wide variety of animals including human, causing trichinellosis. Human infection is caused by ingesting of meat contaminated by encysted infective larvae stage of the parasites. The encysted larvae in the meat maintain the infection for a long time even stored at low temperature (at 4°C in refrigerator) for a long time. Tsdaf-21, *Caenorhabditis elegans* Daf-21 homologues in *Trichinella spiralis*, encodes a heat-shock protein in *Trichinella*, and the cDNA was obtained by RT-PCR. The partial nucleotide sequence of the cDNA has an open reading frame (ORF) of 872 bp encoding a polypeptide of 279 amino acid residues with a HATPase-c superfamily protein domain and HSP 90 protein domain. Phylogenetic analysis of *Tsdaf-21* with HSP90 sequences available from GenBank revealed that *Tsdaf-21* was highly conserved and most closely related to *C.elegans*daf-21. The transcripts of *Tsdaf-21* present in the new born larvae, muscle larvae and adult worm of *T. spiralis*. The Tsdaf-21 was cloned into pET-32m and subsequently expressed in *E. coli*. The recombinant protein TsDAF-21 was purified and evaluated as an antigen by Western blot. The serum collected from mice at 14, 21 and 28 days after infection with *T. spiralis* recognized rTsDAF-21, but the serum of 7-days post infection of *T. spiralis* could not react with rTsDAF-21. It implied that the antibody of TsDAF-21 did appear in the host after infection *T. spiralis* 14 days and could react with rTsDAF-21. The expression of TsDAF-21 in different stages of *T. spiralis* was detected by Western blot. The anti-serum of rTsDAF-21 recognized the 90kDa protein band in the lysates of new born larvae, muscle larvae and adult worms. The immuno-localization showed that TsDAF-21 expressed in the different stages of *T. spiralis,* include new born larvae, muscle larvae and adult worms. And TsDAF-21 was mostly localized in the nuclei of the cell in the worms. These results suggested that worm hsp-90 was highly conserved among the animals and theTsdaf-21 might be a diagnostic marker and candidate vaccine antigen in *Trichinella*.

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47 Production and characterization of monoclonal antibodies against a serine protease from newborn stage of *Trichinella spiralis*

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Serine proteases play important roles at the host-parasite interface and are thought to be involved in host invasion. Some serine proteases have been isolated and characterized from different stages of *T. spiralis* and an enzymatic activity has been identified in *T. spiralis* excretory-secretory (ES) or crude antigens. In previous work, an immunodominant *T. spiralis* serine protease, named NBL1, was obtained by suppression subtractive hybridization cDNA library of *T. spiralis* Newborn Larvae stage (Boireau *et al,* patent 2007). The aim of this work was to produce and characterize monoclonal antibodies (MAbs) against the C-terminal part of NBL1, in order to develop immunodiagnosis tools for pig trichinellosis detection. Two MAbs directed against NBL1 were produced on mice. Briefly, Balb/c mice were immunized twice with the recombinant NBL1-C-terminal. The hybridoma cells producing MAbs were selected after screening by ELISA of supernatant against the recombinant NBL1. The 2 MAbs recognized specifically the recombinant serine protease NBL1 with a unique band at a molecular weight of 43 kDa. Using somatic antigens, a band of 50 kDa was observed with Adult and Newborn mix antigens as well as with Newborn stage alone. On the other hand, both MAbs failed to recognize muscle larvae somatic antigens. Thus, the 50 kDa protein identified represents the native NBL1. Indirect immunofluorescence analysis using cross cryosections of different *T. spiralis* stages revealed that both MAbs intensely stained only the embryos within the gravid females and the cuticle of newborn larvae. The production of these antibodies will allow the development of new immunodiagnosis tools for early detection of *Trichinella* infection in pigs. Moreover, a perspective is open for the characterization of a major antigen of *Trichinella* involved in the invasion of the host.

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48 Towards the first international pig reference serum with anti-*Trichinella* antibodies: isochronous studies on the reference swine serum candidates

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Keywords: *Trichinella*, diagnosis, reference serum, serology

There is a need for reference materials for the serological detection of *Trichinella* infection in pigs to improve the inter-laboratory comparability. Four potential reference sera have been prepared from experimentally infected pigs kept on a specific pathogen-free farm at the EURLP. Moreover two additional sera were collected from *Trichinella*-free pigs (negative controls). Sera were tested, aliquoted, lyophilized, and maintained at +4°C. Since biological products may be sensitive to degradation, the aim was to carry out isochronous studies for stability testing for short and long term, in order to evaluate the possible degradation of the material during transport and storage, respectively. The homogeneity of the samples and their stability at the temperature to which the samples were stored (+4°C), were assumed. For short term stability studies, 2 units were stored at -20°C, +4°C, +20°C, and +50°C for 0, 1, 2 and 4 weeks and then tested 4 times. For the long term stability studies (one year),the same number of units and replicates per unit were taken at -70°C, -20°C, and +4°C, for 0, 4, 8 and 12 months. In both studies, unit samples were selected randomly and tested on the same day under repeatable conditions. It has been accepted that the uncertainty for the measured value should not exceed 3% for a shelf life of 5 years. Data were scrutinized to eliminate any ambiguous result. Furthermore, the regression line versus time for each studied temperature was calculated, and then the slope of the regression line was tested by ANOVA to detect if the trend was statistically significant. In the short term study and among positive sera, a serum (Elisa index 68%; cut off 18%) showed the maximum stability at -20°C, +4°C, and +20°C; moreover, only a 0.6% of degradation (0.6%) was observed at -20°C. Negative sera were stable at +4°C and +20°C; whereas at -20 °C, a small difference between the two control sera was observed. Both the +4°C and +20°C are the temperatures intended to dispatch the serum samples. The long term studies are in progress.

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49 Digestion of individual meat samples for *Trichinella* detection as a substitute of compressor method.

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As compressorium method usage for *Trichinella* detection is no more legal in Europe, the modification of magnetic stirrer arranged for processing small samples became actual for the laboratories performing individual analysis after farm slaughtering in private households. The aim of this study was to evaluate the sensitivity of *Trichinella* larvae for newly designed prototype – multi-reactor apparatus. It includes eight similar reactors mounted on a thermostat, with the possibility to perform the mixing by stirring rods; from a single drive. The volume of each reactor, which is based on plastic syringe with mesh filter inside and magnetic stirrer rod is reduced to 50 ml of gastric juice, that allows to digest meat samples of up to 2.5 grams. After mixing and sedimentation/filtration of *Trichinella* through mesh filter the sediment is evacuated from the syringe by means of a piston, through the needle and into the watch glass. The mixing and sedimentation phase duration, as well as the stabilized temperature are the same as those prescribed in EU Directive 2075/2005 for magnetic stirrer method. Such similarity of physical parameters of digestion process can be of interest for research, for example - to study *Trichinella* larvae distribution in different tissue samples taken from one animal (investigating the distribution of invasion). During tests carried out with the use of tissue samples collected from infected laboratory mice, satisfactory outcome of *Trichinella* was observed, (73-85%). From our point of view the proposed apparatus can substitute the compressor method in the laboratories where the individual tests after group digestion are needed and to become an ideal choice for individually slaughtered pigs, game meat as well as for research institutions.

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50 Development of a serological test to detect the humoral immune response against *Trichinella zimbabwensis* in Nile crocodiles (*Crocodylus niloticus*)

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Keywords: *Trichinella zimbabwensis*, Nile crocodile, serology, ELISA

In order to develop a tool for monitoring *Trichinella zimbabwensis* infection in Nile crocodiles (*Crocodylus niloticus*), serum samples from four experimentally infected and four uninfected crocodiles were obtained after bleeding from caudal tail veins at day 0 and every fortnight for 32 weeks. Moreover, sera were collected from 7 wild Nile crocodiles from Mpumalanga province and from 1 Nile crocodile from a reptile park (South Africa). An indirect ELISA was developed using, as antigens, excretory/secretory products from *in vitro* cultivation of *T. zimbabwensis* larvae according to published protocols. To raise anti-crocodile sera, total Ig were obtained after treatment with caprilic acid of pooled sera from 6 non infected Nile crocodiles. Two New Zealand rabbits were immunized with 50 µg and 100 µg of crocodile total Ig in the presence of Freund adjuvant. Rabbit serum samples were analyzed by immune-electrophoresis versus total crocodile Ig. The most reactive sample was selected to obtain the anti-crocodile sera which were then conjugated with horseradish peroxidase. The conjugated specificity was determined by a direct ELISA using plates coated with serum samples from different animal species (crocodile, dog, horse, rabbit and pig). The anti-sera conjugate was specific to crocodile sera and did not react with the sera of the other animal species. The developed indirect ELISA was used to detect *Trichinella* infection in captive and wild crocodiles; only sera from experimentally infected crocodiles tested positive. Infection intensity in each of the four infected crocodiles was determined from biopsy samples collected from the dorso-lateral muscles of the tail. The highest response was observed 42 days post infection (p.i.) in all of the four infected animals which harbored 7, 36, 2 and 12 LPG, respectively. The earliest antibody response was observed at day 14 p.i. in one animal with 7 LPG. Humoral immune response was detectable only for a short period of time and not after 42 days p.i. The immune response to *T. zimbabwensis* infection in crocodiles appears not to have persisted during the studied time. Serological analysis by ELISA is deemed unsuitable to monitor *T. zimbabwensis* infection in wild crocodiles.

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51 Inspection of *Trichinella spiralis* pre-encapsulated larvae in muscle samples of experimentally infected mice

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Artificial digestion method is widely used to the inspection of *Trichinella* larvae in meat, but it is applied mainly to the examination of the mature larvae (i.g. encapsulated larvae in encapsulated *Trichinella*). Previous studies demonstrated that *T. spiralis* pre-encapsulated larvae at 14-18 days post infection were infective to new hosts. However, to our knowledge, there is no report on the inspection methods of pre-encapsulated larvae in meat. The purpose of this study was to observe the efficiency of artificial digestion method and Baermann's technique for inspection of the pre-encapsulated larvae (PEL) of *Trichinella spiralis* in meat and their affecting factors. Forty-five male Kunming mice were randomly divided into 3 groups (15 mice per group). Each group of mice was orally inoculated with 20, 10 or 5 muscle larvae of *T. spiralis,* respectively. All infected mice were slaughtered at 18 days post-infection and the muscles were cut into pieces. The digestion method recommended by International Commission on Trichinellosis (ICT-digestion), the Chinese criterion "diagnostic techniques for *Trichinella spiralis* in swine" (GB/T18642-2002) (GB-digestion) and Baermann's technique were used to inspect the PEL in the infected muscles. The detection rate of PEL in muscle of mice infected with 20 muscle larvae by ICT-digestion, GB-digestion method and Baermann's technique were all 100% (15/15); The detection rate of PEL in muscle of mice infected with 10 larvae by the above three methods were 93.33% (14/15), 93.33% (14/15) and 100% (15/15), respectively (P>0.05)； When the three methods were applied to examine muscle from mice infected with 5 larvae, the detection rate of PEL were 63.33% (19/30), 90% (27/30) and 100% (30/30), respectively (P<0.05). The muscle of mice slaughtered at 18 days post-infection was digested for 1, 2, 3, 4 and 5h, the death rate of PEL was 8.49% (53/624), 29.77% (181/608), 58.46% (449/768), 67.83% (407/600) and 84.70% (515/608), respectively. The mortality was increased along with prolong duration of digestion (P<0.05). The results shows that Baermann's technique is superior to the digestion method for inspection of *T. spiralis* pre-encapsulated larvae in meat.

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52 Immunochromatographic strip for detection of anti-*Trichinella* antibodies in muscle juice of experimentally infected mice with low level infections

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The aim of this study was to observe the effect of the immunochromatographic strip for detection of anti-*Trichinella* antibodies in muscle juice of experimentally infected mice with low level infections. The strip was prepared by using SPA labeled with colloidal gold and *T. spiralis* muscle larval excretory-secretory (ES) antigens. Seventy male Kunming mice were randomly divided into 7 groups (10 mice per group), each group was orally inoculated with 30, 25, 20, 15, 10, 5, or 3 muscle larvae of *T. spiralis,* respectively. The anti-*Trichinella* antibodies in serum and muscle juice from the infected mice were assayed by the strip 6 weeks post-infection (wpi). The results of strip were compared with those of ELISA, trichinelloscopy and artificial digestion method. In the mice infected with30, 25, 20, 15 and 10 larvae, the antibody positive rate of serum and muscle juice was 100% (10/10) by strip and ELISA, the larvae were detected in 100% (10/10) of muscle samples by trichinelloscopy and digestion method. In 10 mice infected with 5 larvae, the positive rate of trichinelloscopy and digestion method was 70% (7/10) and 100% (10/10), respectively; the larvae were detected in 10 muscle samples with 0.88-3.14 larvae per gram (lpg) (mean 1.58 lpg) by digestion method, the antibody positive rate of serum and muscle juice was also 100% (10/10) by strip and ELISA. In 10 mice infected with 3 larvae, anti-*Trichinella* antibodies in serum and muscle juice were not detected by strip and ELISA and the larvae not found by both trichinelloscopy and digestion method. The results showed the sensitivity of the strip for examination of *T. spiralis* in meat was the same as ELISA and digestion method, and obviously higher than trichinelloscopy（$P<0.05$）. There was a positive correlation between antibody level in muscle juice and infecting dose in mice infected with low dose 6 wpi（$P<0.05$）, the antibody level in muscle juice showed also significant positive correlation with serum antibodies 6 wpi. In conclusion, the mouse muscle samples containing 0.88 lpg could be detected by the immunochromatographic strip and the strip could be used as the preliminary screening test for inspection of meat with low level of *Trichinella* infection.

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53 Detection of anti-*Trichinella* antibodies in serum of experimentally infected swine by Immunochromatographic strip

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The immunochromatographic strip developed with the excretory-secretory (ES) antigens of muscle larvae of *Trichinella spiralis* and labelled with colloidal gold was used for the detection of anti-*Trichinella* antibodies in serum of experimentally infected swine. Serum of swine infected with 200, 2000 and 20000 infective muscle larvae were collected at different days post infection (dpi) and used to evaluate the strip. The strip was shown to be able to detect the anti-*Trichinella* antibodies at 35 dpi, 28 dpi and 21 dpi for the different infection doses, respectively, and which were closely correlated with those of ELISA. The dipstick assay based on the strip is rapid and easy to perform and is suggested as an acceptable alternative for use in clinical laboratories lacking specialized equipment, as well as for field diagnosis of trichinellosis.

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54 Application of liquid gene chip technique for detecting main food-borne parasites

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Keywords: liquid gene chip, parasitic zoonoses, *Toxoplasma gondii*, *Trichinella spiralis*, *Cysticercus cellulose, Clonorchis sinensis*

Four pairs of primers and probes were designed according to *Toxoplasma gondii* B1, *Trichinella spiralis* 18S rDNA, Cysticercus cellulose ITS1 and *Clonorchis sinensis* ITS1 gene sequence, respectively. Using genomic DNA as the template, four short gene fragments were simultaneously amplified by multiplex PCR. The products of the multiplex PCR and the single PCR were detected by the liquid gene chip technology. The results showed that the sequence of *T. gondii* B1, *T. spiralis* 18S rDNA, *C. cellulose* ITS1 and *C. sinensis*ITS1 were 188 bp, 92 bp, 154 bp and 170 bp. The nucleotide homologies were 99.47%, 100%, 99.35% and 98.27% which compared with their corresponding sequence published in GenBank. The reproducibility of the test showed that each of CV % of liquid gene chip was less than 7.3 %. The detection limit of the liquid gene chip technique was 65.6 ng/mL, 53.52 ng/mL, 87.89 ng/mL and 39.06 ng/mL for *T. gondii, T. spiralis, C. scellulose* and *C. sinensis*, respectively, which was 8-fold more sensitive than that of agarose gel. The simulation of pollution test showed that the accuracy rate of blind test more than 93%. Finally, we used four kinds of diagnostic microspheres for four parasites. This new technology, which was more stable, specific and sensitive than PCR and multiplex PCR could be valuable for simultaneous detection of the four parasites by one reaction in a single vessel within 3.5 h. In addition, liquid gene chip technique is a rapid, sensitive and specific method for detecting *T. gondii, T. spiralis, C. cellulose* and *C. sinensis* in food.

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55 Epitopes scanning of newborn larvae stage-specific antigenic gene T668 in *Trichinella spiralis*

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Keywords: *Trichinella spiralis*, antigenic epitope, antigen-specificity, immunoblotting

It is very necessary to develop early stage diagnosis reagent of *Trichinella spiralis* and important to find a gene which has high antigenicity and specificity. NBL stage-specific Gene T668 is such a high antigenic gene screened from cDNA library of NBL of *Trichinella spiralis* from our lab. Though it is a high reactogenicity gene, it has significant cross-reaction with the serum obtained from healthy swine. We screened its epitope in order to detect its antigen-specificity. Here, we analyzed the hydrophilicity, antigenicity and surface accessibility of T668 sequence (GeneBank ID: AF331160.1) by DNAstar software, then divided it into 4 regions which were called His, Asp, Ser and hydrophilic domain in C-terminal referred to as T668H. After prokaryotic expression and purification, we identified that T668H region was a major epitope determinant domain by immunoblotting and ELISA. To map the antigenic epitopes of this region, we designed 16 short peptides with 5 amino acids frame shift superposed among this domain named $N5EM_1$-$N5EM_{16}$, and then fusion expression with GST in a pGEX-4T-1 vector. Immunoblotting and ELISA results demonstrated that $N5EM_3$, $N5EM_4$, $N5EM_5$, $N5EM_7$, $N5EM_8$, $N5EM_9$ and $N5EM_{11}$ had high response signals with swine-to-*Trichinella spiralis* antiserum, but their response signals with negative serum displayed obvious either. It is because that GST has cross-reactivity with negative serum. Based on the results above, the seven short peptides were artificially synthesized and coupled with bovine serum albumin (BSA). After dot blotting and ELISA analysis, we finally screened $N5EM_{11}$ as the dominant epitope. This result should help guide functional studies and further epitope-based immunological diagnosis.

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56 *Trichinella*: What's going on during nurse cell formation?

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Keywords: *Trichinella*, nurse cell, satellite cell, proliferation, differentiation, transformation, apoptosis

Trichinella infection causes profound changes in host muscle cells, resulting in formation of a capsule in infected muscles, known as nurse cell. The process of nurse cell formation is complex. The response of infected muscle cell at early stage is similar to that occurring in myogensis and muscle regeneration, including the activation, proliferation and differentiation of satellite cell and cell cycle re-entry. At the late stage of nurse cell formation, development of infected muscle goes along with the demands of larva, for example, arrest of cell cycle, the change of basophilic and eosinophilic cytoplasm, involvement of apoptosis and anti-apoptosis, and transformation of infected muscle cell. Corresponding these processes, several genes or signaling pathways have been identified to be involved in the nurse cell formation, for example, myogenin and MyoD in satellite cell activation, TGF-beta signaling pathway in cell cycle arrest and transformation, TNF-alpha and mitochondrial mediating apoptosis, IGF signaling pathway in cystogenesis, and IR signaling pathway in glucose metabolism. Therefore, it could be proposed that the process at the beginning is a response of host cell to larval invasion, while the process at a later stage it is reforming or restructuring of host cell processes by larva. On the other hand, how the *Trichinella* larva reforms muscle cell into its home is an interesting issue but still unknown. It is proposed that the parasite takes advantage of the host cell biological system to build its home via its products, known as parakines, as messengers to carry out the molecular cross-talking between parasite and host cells. Further study of the roles of the parasite products will be an important subject in revealing the mechanism of nurse cell formation.

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57 Analysis of the effect of the new benzimidazole derivate GNV14, on *Trichinella spiralis* muscle larvae

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Keywords: *T. spiralis*, benzimidazole derivative, proteomic analysis

Benzimidazole 2-carbamate derivatives, such as albendazole (ABZ) and mebendazole (MBZ) are widely used in the chemotherapy of human and animal trichinellosis. These compounds have a high therapeutic index and low toxicity; however, high doses and long treatments are required for the treatment of systemic trichinellosis mainly due to their poor solubility and bioavailability. In fact, chemotherapy failure at the muscle larvae (ML) phase has been reported in patients treated with MBZ or flubendazole. In an effort to obtain new anthelminthic compounds we synthesized the benzimidazole derivative, 5-(2,3-Diclorofenoxi)-1-metil-2-(trifluorometil)-1*H*-benzimidazol, named GNV14. The *in vitro* activity of GNV14 against *T. spiralis* muscle larvae (ML) was determined by a colorimetric method based on the reduction of MTT. In this study, GNV14 was more active than the reference compound ABZ (80% versus 67 %, respectively). The *in vivo* activity of GNV14 in inclusion with 2-Hydroxypropyl-β-cyclodextrin (GNV14/HPβCD) against *T. spiralis* was determined using a mouse model; the inclusion complex reduced 84% the ML load in comparison to 31% induced by ABZ alone. In addition, the acute toxicity of GNV14, tested in mouse, was higher than 500 mg/body weight and no cytotoxicity in cell line MDCK was detected at 10 μg/mL. In order to analyze the mechanism of action of this new benzimidazole derivative, a proteomic analysis of *T. spiralis* ML treated with GNV14 at 1 μg/mL was performed. The 2D analysis (pH range 3-10 and 4-7) of total extract obtained from ML treated with GNV14 showed the differential expression of at least 24 protein spots in comparison with the untreated parasite; 6 spots showed an increase in expression and 18 spots have a decrease in expression in the treated sample. The protein spots were selected and eighteen of them were identified by Mass Spectrometry.

The ultrastructural effect of GNV14 on ML analyzed by transmission electronic microscopy (TEM) and scanning electronic microscopy (SEM) revealed major changes at the cuticle, hypodermis and muscular level of the parasite.

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58 A new formulation of mebendazole in low-substituted hydroxypropylcellulose (L-HPC): improved efficacy against experimental trichinellosis

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Key words: mebendazole, hydroxypropylcellulose, solid dispersion, Swiss-CD1 mice, *Trichinella spiralis*

Mebendazole (MBZ) has been used as first choice drug for treatment against trichinellosis. However it anthelmintic effectiveness is compromised by its poor water solubility which limits the drug bioavailability in biological fluids as well as by its undesired side effects such as teratogenicity. To overcome these problems various technological resources have been explored such as the preparation of solid dispersions, liquid solutions or cyclodextrin complexes with different degree of success. Here a new formulation consisting on preparation of solid dispersions of MBZ in different proportions of low-substituted hydroxypropylcellulose (L-HPC) is evaluated against successive enteral and parenteral stages of *T. spiralis* infection in mice. Female Swiss-CD-1 mice of 30 g body weight were orally infected with 300 L1 larvae of *Trichinella spiralis.* At different times along the parasite life cycle : day 1 or day 6 post-infection (p.i), against intestinal pre-adult and adults stages respectively; days 13, 14 and 15 p.i., against migrating new born larvae (NBL) and days 33, 34 and 35 p.i. against encapsulated muscle larvae, the corresponding groups of animals were treated with the new formulation at a daily dose ranging between 1 and 5 mg/kg in parallel with a classical dispersion of the primary drug in carboxymethylcellulose (CMC) similarly given to age-matched infected animals. The efficacy of MBZ under the new formulation was significantly increased against all stages on *T. spiralis* infection and particularly against pre-adults and encapsulated muscle larvae were the percentage of reduction regarding untreated controls were 87.91% and 97.36% versus 19.80% and 21.36% achieved with the primary drug, respectively. The pharmacokinetic studies showed that the bioavailability of MBZ is near three fold increased when administered as solid dispersion compared to the conventional dispersion in CMC. Complementary studies carried out on physical characteristics and dissolution rate of the new formulation indicate that the increased solubility of MBZ solid dispersion may be attributed to the transition of the drug from the thermodynamically stable crystalline form to a more unstable amorphous stage. In conclusion this new formulation may be suitable to improve the efficacy and safety of MBZ in treatment against trichinellosis.

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59 TGF-beta and hedgehog signalling pathways in *Trichinella spiralis*

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Keywords: transforming growth factor-beta, Hedgehog, *Trichinella spiralis, Caenorhabditis elegans, Prionchulus punctatus*

The recent release of genome sequence information is revolutionising the study of helminth parasites by providing important datasets for comparative genomics that will allow us to analyse the signalling pathways that regulate nematode parasite development. Much of our current knowledge of nematode signalling pathways is based on the study of the free-living model *Caenorhabditis elegans*. While most are conserved (e.g. TGF-beta pathway), a number of pathways are missing in *C. elegans*, e.g. the JAK/STAT signalling pathway, or are incomplete. Most strikingly, the hedgehog signalling pathway in *C. elegans* is modified and lacks a bone-fide Hedgehog ligand. Studies indicate that other clade II, IV and V nematodes (e.g. *Brugia malayi*) are likely to be similar to *C. elegans*. The availability of the genome sequence for the clade I nematode *Trichinella spiralis* has allowed us to study signalling pathways in a basal nematode. We have undertaken an analysis of the hedgehog and TGF-beta signalling pathways in *T. spiralis* and have identified a gene encoding a bone-fide hedgehog ligand. In addition we have identified 5 genes encoding TGF-beta-like ligands, three of which belong to the BMP subfamily and are closely related to the vertebrate BMP2/4, BMP5/8 and BMP3 proteins. The remaining two proteins clearly belong to the TGF-beta/activin subfamily. To date only one member (DAF-7) of this subfamily has been identified in other nematode species, including *C. elegans*. Our recent analysis of another clade I nematode, *Prionchulus punctatus*, is providing additional insights into the biology of basal nematodes.

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60 Antigen genes display of *Trichinella pseudospiralis* at different development stages

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Keywords: *Trichinella pseudospiralis*; different development stages; antigen genes; immuno-screening.

Trichinella psudospiralis (*Tp*) differs from *Trichinella spiralids* (*Ts*) in interesting of parasitological and pathological characteristics. Besides the obvious difference of capsule formation, they also differ in satellite cell response. The differences between *Tp* and *Ts* also extend to aspects of the host's immune and inflammatory responses to infection. In both primates and rodents, *Tp* is less pathogenic than *Ts*, generating considerably less inflammation in the intestine and muscles of the host. To investigate the different antigens talking between the host and the parasite of two species is obviously significant not only for understanding their different pathogenic mechanism but also for diagnosis, prevention and treatment, whereas more works were focused in *Ts* but few understanding for *Tp*. In this work, antigen genes of *Tp* at different development stages were displayed by immune-screening the different stage larvae's cDNA libraries. The expression cDNA libraries of *Tp* adult (Ad, 3 days old adult), newborn larvae (NBL) and muscle larvae (ML) were constructed and screened by the pig anti-*Tp* serum on 26, 32 and 60 dpi, respectively. Two high abundant (22 and 23 of 69 total positive clones, respectively) antigen genes were found from *Tp* adult cDNA library, which encoded the similar proteins of 6-phosphogluconolactonase [Bombyx mori] and putative pyroglutamyl-peptidase 1 [Trichinella spiralis] with similarities E-value 3e-70 and 9e-44. Interestingly all the high abundant antigen genes found in NBL and ML were stage-specific, they are the newborn larvae-specific serine protease SS2-1 [*Trichinella spiralis*] similar (E-value 0.0) gene with abundance 10/24 from NBL cDNA library, and the similar (E-value 7e-115, 1e-91 and 3e-123) genes of putative proteasome activator complex subunit 3 [Trichinella spiralis], 21 kDa excretory/secretory protein [Trichinella pseudospiralis] and 28 kDa excretory/secretory protein [Trichinella pseudospiralis] with abundances 11/26, 6/26 and 3/26 respectively from ML cDNA library. The potential bio-functions and applications of these antigen genes in *Tp* infection were discussed.

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61 *Trichinella spiralis* and *Caenorhabditis elegans* display a parallel pattern of thymidylate synthase localization

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T. spiralis and *C. elegans* are two nematode species, representing life styles of an intracellular parasite and free-living worm, respectively. Our previous studies documented thymidylate synthase, an enzyme associated with DNA synthesis constituting (based on studies on mammalian cells) a marker of proliferating or cell cycle-arrested cells, to be highly expressed in larvae, including developmentally arrested, and adult forms of both species. In the present study confocal microscopy was applied to follow the enzyme localization during development of both species. In regard to both species high thymidylate synthase level was detected in both reproductive and excretory-secretory systems of larval forms, in gonads and embryos developing in the uteri, as well as excretory-secretory systems of adult forms. While high enzyme level found in developing embryos appears by virtue of embryonic development to be associated with cell proliferation, that found in reproductive and excretory-secretory systems is proposed to be associated with cells of those systems being cell cycle-arrested. In *T. spiralis* muscle larva and adult forms, in excretory-secretory organ stichosome, thymidylate synthase was localized to nuclear region of stichocytes, canalicula and was also found lining esophagus wall. The latter implicated its putative secretion and antigenic properties. However, the enzyme was not detected in the nurse cell space surrounding the larva. As thymidylate synthase was also found expressed at high level in *C. elegans* secretory system, its presence in excretory-secretory cells appears specific for phylum nematoda rather than arising from parasitic life style of *T. spiralis*.

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62 Freezing resistance of *Trichinella* muscle larvae in wild boars experimentally infected

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Keywords: *Trichinella britovi*, freezing treatment, wild boars experimentation

Freeze tolerance of encapsulated *Trichinella* muscle larvae (ML) is influenced by *Trichinella* species, host species and also the age of the infection. Indeed, longer is the infection, thicker is the capsule which protects *Trichinella* ML and could thus allow the parasite to be more resistant to freezing processes. Moreover, *T. britovi* hosted in pig is inactivated by freezing treatment at -17°C for 1 week, even when infection is as old as 41 weeks. Nevertheless, 6 human trichinellosis cases were reported following the consumption of wild boar meat frozen 7 days at -35°C [(1)].The incriminated species was *T. britovi*. In addition, it was showed that *T. britovi* encapsulated in wild boar was still able to infect host after 3 weeks at -20°C; 4 weeks at -20°C were required to inactivate larvae [(2)]. This study focused on freezing resistance of *T. britovi* isolated from long-term infections in wild boars. Three groups of 4 8-weeks old wild boars were infected with 200, 2,000 or 20,000 ML of *T. britovi* (ISS 1575). An additional group of 3 wild boars were inoculated with 20,000 ML of *T. spiralis* (ISS 004) and 2 wild boars were included as negative control. All wild boars were sacrificed 24 weeks post infection and biceps brachii were removed from carcasses. Muscle samples of 70g were stored at –21°C for 19h, 30h, 56h and for 1 to 8 weeks. Larvae were recovered by artificial digestion and their mobility was recorded using the Saisam® image analysis software. Infectious capacity of larvae was evaluated after inoculation in mice. Movements of ML were observed after digestion of samples frozen for 19h, 30h and 56h but not for 1 and 2 weeks. Furthermore, mice infected with *T. spiralis* and *T. britovi* larvae from pork frozen for 19h, 30h and 56h hosted ML but mice were negative if longer freezing times were applied. This study showed that freezing treatment at –21°C for 1 week inactivated *T. britovi* larvae encapsulated in wild boar meat up to 15 cm in thickness for an infection as old as 24 weeks.

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63 Serine proteinase inhibitor (Serpin)-A potential good diagnosis target for *Trichinella* late infection

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Serine proteinase inhibitors (serpins) play a vital regulatory role in a wide range of biological processes, and serpins from viruses and nematodes have been implicated in pathogen evasion of the host defense system. In our previous work, a high frequency cDNA clone, designated WM5, encoding a high antigenic *Trichinella spiralis* serine proteinase inhibitor (*Ts-serpin*) was obtained from a muscle larvae cDNA library by immunoscreening with pig anti-*Trichinella* sera obtained at 60 days post infection (dpi). However, there is still a dearth of publications available for in-depth research concerning this functional gene. In this work, *Ts-serpin* was cloned and identified as a stage-specific antigen. Real-time RT-PCR revealed that the *Ts-serpin* gene was transcribed in every developmental stage of *Trichinella spiralis* but strictly in muscle larvae and adult worms. Using immunohistochemistry, we found that native *Ts-serpin* was localized mainly in worm bodies and specifically in the nucleoplasm of the host muscle cell's enlarged nuclei from 20 dpi. to 30 dpi. The recombinant protein r*Ts-serpin* could be recognized by pig sera at 60 dpi. *Ts-serpin* played an important role in the entire nematode life cycle, especially in the muscle larvae stage of *Trichinella spiralis*, and may be a good candidate for diagnosis, especially in the later stages, of trichinellosis.

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64 Potential roles of insulin-like growth factors in different pathology of infected muscle cells between *T. spiralis* and *T. pseudospiralis*

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Keywords: *Trichinella spiralis, T. pseudospiralis*, IGF, IGF binding protein, nurse cell

Trichinella spiralis and *T. pseudospiralis* induce different pathology during the development of the infected muscle cells. It is still unknown what kind of molecular mechanism is corresponding to the formation of nurse cells and the different pathology between the two species of *Trichinella*. The insulin-like growth factors (IGFs), their receptors, and binding proteins constitute a family of cellular modulators that play essential roles in the regulation of growth, differentiation and survival, as well as metabolic processes. In the present study, we investigated the expression and the kinetics of IGF signaling related genes, including IGF-I, IGF-II, IGF-IR, IGFBP2, IGFBP4 and IGFBP5, during development process of nurse cell formation and compared the expression difference between *T. spiralis* and *T. pseudospiralis* infection. The results indicated that after infection, both *Trichinella* induced the increased expressions of these IGF signal related genes, although the expression kinetics was different between them. Moreover, there were markedly different in the expressions of IGFBP2 and IGF-II between *T. spiralis*- and *T. pseudospiralis*-infected muscle cells. *T. spiralis* infection caused extreme increase in the expression of IGFBP2 in the infected muscle, as high as 442 folds compared with that in normal muscle cells, while *T. pseudospiralis* infection caused some degree increase (4-7 folds), much lower than that in *T. spiralis*. Immunohistochemical analysis revealed that the increased expression of IGFBP2 was mainly limited in the enlarged nuclei within infection muscle cells. The present study suggests that IGF signaling pathway plays important roles in the nurse cell formation, and IGFBP2 may be key factor to contribute to the different pathology between the two species of *Trichinella* infection, as upstream regulators in the proliferation, differentiation, transformation and apoptosis occurred in the infected muscle cells.

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65 Morphometric and molecular characterization of early intestinal stages of *Trichinella spiralis*.

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Keywords: *Trichinella spiralis*, preadults, early adults, morphometry, cDNA libraries

Synchronisation of *Trichinella* early stages is not possible during the intestinal phase of infection. Indeed, muscle larvae (ML) can be found up to 6 or 8 hours post infection (hpi) in the intestinal tract of experimentally infected mice. So in the attempt to purify early stages of maturation up to Adult stage, it was necessary to avoid the presence of ML in the parasite purification. A first deadline for early stages purification was thus selected at 14 hpi. Two additional times (20 hpi and 48 hpi) were added to overlap all the moults up to the adult stage. In order to characterize the relevance of these chosen times of infection, morphometric and molecular characterization of these worm populations were performed. After recovery of worms from mice intestines for the three deadlines mentioned above, measurements of length and width of parasites were performed using the Saisam® software. Measurements of ML were also performed as reference. Data were then analysed by a linear mixed model using the R software. Expression of various genes previously described was checked using PCR on cDNA for these three periods of *Trichinella* development. Morphometric study showed a lengthening of the worms from ML to 48 hpi. Statistical analysis revealed a significant difference in length between 14 and 20 hpi populations, and significant differences in width between ML and 14 hpi and between 20 and 48 hpi parasites. Moreover, sexual organs (male and female) were observed on worms collected at 48 hpi. Nevertheless, embryos were never observed in the female womb, indicating that worms at 48 hpi are young adults. Among genes expression tested, AdTs1 was expressed in AdD3 while it was not in ML, as previously described. As suspected, AdTs1 was expressed in 48 hpi parasites, in which fertilized eggs could have divided. Surprisingly, AdTs1 was also expressed in 14 and 20 hpi worms despite those populations were not sexually mature. Alignment of the nuclear receptor sequence of AdTs1 with other parasites receptor sequences involved in moulting showed relevant identity. This could explain the expression of AdTs1 over intestinal phase during which *Trichinella* undergoes several moults, unlike the ML stage. Results of the morphometric study allowed the characterization of parasites recovered 14h, 20h and 48 hpi as three distinct populations and the updating of data on intestinal stages of *Trichinella*. At last, AdTs1 could be the first marker of moulting described for *Trichinella* genus.

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66 Rcd1 protein secreted from *Trichinella spiralis* down-regulates myogenin and MyoD proteins in C2C12 myoblasts, and inhibits binding activities of transcription factors NF-κB and AP-1.

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Keywords: *Trichinella spiralis*, myoblast, differentiation, Rcd1, myogenesis, NF-κB signaling pathway

Trichinella spp. infection causes satellite cell proliferation and transformation of muscle cell to the nurse cell. Rcd1 (Required cell differentiation 1) initially identified as a factor essential for the commitment to nitrogen starvation-invoked differentiation in fission yeast, is one of the most conserved proteins found across eukaryotes, and is shown to be a transcriptional cofactor of the c-myb proto-oncogene product. Previously, we cloned Rcd1 gene from the cDNA of *T. spiralis* muscle larvae, and confirmed that the Rcd1 protein is part of the E-S products, and is secreted by muscle larvae before stichosome formation into the host cell. Therefore, the Rcd1 likely affects host cells and tissues for capsule formation. Terminal differentiation of myocytes involves withdrawal from the cell cycle, induction of myogenic regulatory factor myogenin and MyoD expression, and formation of myotubes. And transcription factors NF-κB and AP-1signaling are implicated as an important regulator of skeletal muscle homeostasis. In the present study we investigated the effect by which the Rcd1protein secreted from *T. spiralis* modulates myogenic differentiation in C2C12 myoblasts, and the role of the Rcd1 protein regulated NF-κB and AP-1 transcriptional pathway. The kinetics of the myogenin gene expression in C2C12 myoblasts increased at 3 days after the transfer of cells to differentiation medium, and reached a peak at 7 days after differentiation stimulus, and the MyoD gene was expressed in C2C12 myoblasts before differentiation stimulus, and reached a peak 5 days after differentiation stimulus. The expression of myogenin and MyoD genes in C2C12 myoblasts transfected with pcDNA3.1-Rcd1 vector was significantly lower than those in C2C12 myoblasts transfected with pcDNA3.1 empty vector. Transfection of pcDNA3.1-Rcd1 vector in HEK293 cells resulted in the inhibition of binding activities of NF-κB and AP-1. In this study we demonstrate that Rcd1 is a potent inhibitor of muscle differentiation, and can inhibit activation of NF-κB and AP-1signaling pathway. Transcriptional initiation of eukaryotic genes depends on the cooperative interaction of various transcription factors. It may be inferred from these results that the Rcd1 protein functions as a transcriptional factor or cofactor of satellite cell proliferation and muscle cell transformation.

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67 Carriage of *Trichinella* as a form of symbiotic relations

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Keywords: *Trichinella*, biopreparation, cellular immunity, biocenosis, mutualistic symbiosis

In the nature there is nothing absolutely useful or harmful. The world is paradoxical and this is one of the fundamental laws of its existence. Nevertheless parasitologists usually discuss only one, a harmful side of parasitism. However on the basis of the laws of dialectics and first of all the law of unity and fight of opposites one should propose that there is also a useful for a master organism side of parasitism. Parasites of the master are alien as a rule and to their antigens an immune answer is worked out by the master. Parasites are the only suppliers of such a kind of antigens. Consequently to keep up immune defense on a high level a master has to periodically get different parasites (virus, bacteria, and helminths) otherwise its function dies down. *Trichinella* are evolutionary tens of millions of years older than a man, well adapted to animals with chiefly meat type of feeding and should be considered as co-members of any biocenosis. On the one hand *Trichinella* are somehow without a doubt regulators of the number of their masters. On the other hand *Trichinella* carry out diverse functions providing their master with survival and prosperity. Having powerful antigenic complexes *Trichinella* stimulate an immune system of the master, keep it up on a high level and consequently promote the main task of immunity – defense of the genetic consistency of the inner environment of the organism in the constantly changing world. In such a way *Trichinella* as a co-member of biocenosis play an important role in ecological systems and they can be characterized as mutualistic symbiontes. Developing this idea we created from *Trichinella* a biopreparation ("Britov's vaccine') which is able to induce into the organism of warm-blooded animals and a man cellular immunity of high tension. The method is patented in Russian Federation Nr.2172182 (August 20, 2001). Thanks to unspecification of cellular immunity the method turned out to be effective for premature ageing, different gastro-intestinal diseases, diseases of locomotive apparatus, as well as respiratory, hematogenesis, reproductive and urogenital disorders. High effectiveness of the bio preparation (up to 90%) is marked at monotherapy of tuberculosis, papillomavirus infection and infectious hepatitis, HIV-infection, pretumor conditions and cancer of the first and second stages of different localizations. High effectiveness, technology, safety, simplicity of the use of "Britov's vaccine" open in our opinion wide perspectives for its practical application in prevention and treatment of somatic and infectious diseases of a man and animals.

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68 Identification and Characterization of Deoxyribonuclease in Excretory/Secretory Products of *Trichinella spiralis*

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Keywords: *Trichinella spiralis*, deoxyribonuclease, zymographic analysis, NanoLC-ESI-MS/MS

Deoxyribonuclease activity was investigated in excretory/secretory (ES) products of *Trichinella spiralis* infective-stage larvae and adults/newborn larvae, respectively. Both ES products from infective-stage larvae and Adults/Newborn larvae show prominent deoxyribonuclease activity in acidic buffer (pH 5.0) and relative mild endonucleases activity in alkalescent buffer (pH 8.0). The acid deoxyribonuclease activity was divalent cations-independent while the alkalescent deoxyribonuclease activity could be abolished in present of EDTA. Active Gel analyse reveal several bands contributed to these deoxyribonuclease activities, including 67kDa, 58kD, 30kDa, 24kDa, 17kDa bands in infective-stage larvae ES products and 50kDa, 33kDa, 24kDa, 15kDa bands in adults/newborn larvae ES products. Dependency of cation/pH and sensitivity to DNase inhibitors of each band was examined by zymographic analysis. The activity at 67kDa in infective-stage larvae ES products require acidic pH and sensitive to EDTA, which show different characteristics to classic acidic DNase. The 17kDa and 15kDa polypeptide bands were active in either acidic or alkalescent condition, suggesting their unusual properties when compared with classic DNase I and DNase II. All the active bands were recovered from the active gels and the potential deoxyribonucleases were indentified by NanoLC-ESI-MS/MS peptide sequencing technology.

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69 Inhibition of Mammalian Muscle Differentiation by Excretory/Secretory Products from *Trichinella spiralis*

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Keywords: *Trichinella spiralis*, excretory-secretory products, differentiation, C2C12 Myoblasts

The excretory-secretory products (ESP) released by *Trichinella spiralis* (*T. spiralis*) was suggested to be involved in nurse cell formation. However, the molecular mechanism by which ESP modulate the nurse cell formation is still unclear. In the present work, utilizing a C2C12 myoblast cell line, the ability of ESP from muscle larvae of *T. spiralis* to influence proliferation and differentiation of murine myoblasts was evaluated *in vitro*. The results showed that ESP enhanced myoblast proliferation and the expression of a cell-cycle regulator cyclin D1 and PCNA. Conversely, ESP reversibly inhibited their differentiation evident by a reduction in the level of MHC and MRFs (MyoD and Myogenin) expression. Taken all together, these results imply that the ESP are able to modulate the murine muscle proliferation and differentiation, which provide an important insight for full understanding of the mechanisms of *T. spiralis* to induce muscle cell phenotype change.

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70 Characterization of a High Frequency Gene Encoding a Strongly Antigenic Cystatin-like Protein from *Trichinella spiralis* Muscle Larvae in the Earliest Intestinal Stage

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The intestinal stage is the earliest invasion stage of *T. spiralis*, in which the decapsulated muscle larvae enter in intestine epithelial cells interacting with the new host, and then develop to adult worms to breed newborn larvae. Antigens from parasites of this initial pathogen-host interaction stage may aid early diagnosis of trichinellosis and may hold promise in developing vaccines to prevent such infections. In order to identify the antigens involved in this stage of *T. spiralis*, a cDNA library of intestinal muscle larvae that just penetrate into intestine epithelial cell at 6 hours post-infection was constructed and immunoscreened with anti-*T. spiralis* serum collected from pigs 26 days p.i. A strongly reactive clone, represented frequently in this library, , encoded a novel cystatin-like protein of *T. spiralis* (Ts-CLP). Real-time PCR revealed that *Ts-CLP* gene was transcribed in every developmental stage of *T. spiralis*, but most especially in intestinal muscle larvae, muscle larvae, and adult worms. Native Ts-CLP was localized mainly in the parasite's β-stichocytes. The recombinant protein rTs-CLP induced protective immunity in mice and could be recognized by 26 days p.i. sera of *T. spiralis*-infected mice, rabbits, and pigs. These data indicate that Ts-CLP probably plays an important role throughout the life cycle, especially in the early infection, and may be a good candidate for early diagnosis and immunoprotection of trichinellosis.

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71 The effects of Nitazoxanide on *Trichinella spiralis in vitro* and *in vivo*

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Keywords: Nitazoxanide, *Trichinella spiralis*

Nitazoxanide (NTZ) is a novel broad-spectrum intestinal antiparasitic drug. However, there is no more information about its effect on helminths such as *Trichinella* sp. In the present study, we investigated the effects of Nitazoxanide on the viability of *Trichinella spiralis in vitro* and *in vivo*. The *Trichinella spiralis* muscle larvae were incubated with NTZ at concentrations varying from 2, 10, 25, 50, 100, 180 and 250 μg/ml for 4 hours *in vitro*. The death or alive of the parasites were determined by Laser Scanning Confocal Microscope. The results showed that the muscle larvae in 250 μg/ml and 180 μg/ml NTZ groups were all died, but 83% muscle larvae was death in 125 μg/ml NTZ group. However, the other groups were alive. The effectiveness of NTZ on different stages infected by *Trichinella spiralis* in mice was also examined. After infected by *Trichinella spiralis*, the mice were orally administrated by NTZ at 25 mg/kg, 50 mg/kg and 100 mg/kg body weight at second, third and 15th, 16th day respectively. The mice were necropsy at 7th and 35th and parasites were calculated respectively. The results showed that the adults were reduced by 13%, 27% and 87% in 25 mg/kg, 50 mg/kg and 100 mg/kg body weight groups respectively. There was no significant effect on migrating larvae (P>0.05). Our results suggest that NTZ have more effect on muscle larvae *in vitro* and intestinal stages of *Trichinella spiralis.*

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72 Sequencing the genome of *Trichinella spiralis*: learning from the past

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Keywords: *Trichinella spiralis*, genome, sequence, nematode, evolution

In 2004, the National Human Genome Research Institute, an integral part of the National Institutes of Health, initiated a large scale genome sequencing effort to better understand metazoan evolution. Among the organisms to be studied was the nematode, *Trichinella spiralis*, a food-borne zoonotic parasite of worldwide importance and distribution, and a nematode associated with early radiation of the phylum. The nuclear genome of *T. spiralis* is the first to be sequenced from a basal lineage within the nematoda. The *T. spiralis* draft genome encodes nearly16, 000 proteins, spans 64 million base pairs and was derived from a 35-fold coverage using whole-genome shotgun and hierarchal map-assisted sequencing. Comparative studies involving genome sequences from more recently diverged nematode lineages as well as the arthropod *Drosophila melanogaster* revealed multiple intriguing trends that will require further scrutiny as more nematode genomes come available. Results suggest that gene loss and gain events among nematoda exceed those observed within the arthropoda, and that protein family deaths surpass births when comparing a parasitic to a non-parasitic nematode; intrachromosomal rearrangements were observed throughout the phylum. Within this analysis, archetypical genes of potential evolutionary importance along with pan-phylum characteristics exclusive to all nematodes were identified. This genome sequence along with the nematode specific molecular signatures identified within this study make available resources to evaluate nematode and metazoan evolution, and provide foundations for developing new strategies to treat and/or eradicate important parasites of humans and livestock.

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73 Shared and disparate patterns of developmentally regulated gene expression in two agents of human trichinellosis.

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A comparative framework can be very useful in establishing the function and importance of gene products in human pathogens, most especially for pathogens only remotely related to model organisms. The principal agent of human trichinellosis, *Trichinella spiralis*, is one such organism whose just-sequenced genome will promote biological insight and clinical progress only in the light of additional comparative data. We therefore sought to compare its gene expression in three life history stages of its enigmatic congener, *T. pseudospiralis*. To do so, we generated 54485 high-quality ESTs for three life stage of *Trichinella pseudospiralis*. Our data supply substantial new insights to the biology of this species, the process by which it forms nurse cells, its means of interacting with host cells and evading immunity. Furthermore, we identified a core of shared and constitutively expressed 'housekeeping' genes that evidently are essential to basic parasite metabolism, as well as elucidated genes and gene families that are differentially expressed in particular parasite species and life history stages. In particular, we identified a family of DNase II proteins, metalloproteases, and genes that may disrupt host cell cycle regulation via histone acylation as potentially important aspects of the parasite's interaction with its mammalian hosts. These data furnish many potential antigen targets that are specific to particular stages of each of these parasites.

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74 Molecular identification of nematode larvae different from those of the *Trichinella* genus detected by muscle digestion

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Keywords: nematodes, *Trichinella*, molecular identification, artificial digestion.

One of the tasks of the EURLP is to identify nematode larvae detected in muscles of domestic and wild animals by digestion. About 400-500 *Trichinella* sp. isolates are identified at the EURLP each year, but sometimes larvae do not belong to the genus *Trichinella*. Often these nematodes are enough different in size and shape from a *Trichinella* sp. larva to exclude the risk of a glaring blunder; however sometimes, it is very hard to identify these false *Trichinella* larvae by a microscope, because of the absence of peculiar morphological characters or of damages of the external cuticle. Consequently, the only possible approach to identify the larva is by PCR and sequencing of specific molecular markers. From 2008 to 2011, 15 nematode isolates not belonging to the genus *Trichinella*, have been analysed (about 1% of those analysed as *Trichinella* spp. in the same period). Eight of these isolates have been successfully identified at the species, genus or family level by using four molecular markers (12S mtDNA; COI; 18S rDNA; ITS1). Nematode larvae isolated from two common kestrels (*Falco tinnunculus*), a hen harrier (*Circus cyaneus*) and a domestic pig (*Sus scrofa*), were identified as *Toxocara cati;* larvae from a badger (*Meles meles*) were identified as *Toxocara canis*; larvae from a domestic pig were identified as belonging to a free living nematode of the genus *Panagrolaimus*; larvae from a wild boar (*Sus scrofa*) were identified as belonging to the *Metastrongylus* genus; and larvae from a rough-legged buzzard (*Buteo lagopus*) were identified as belonging to the superfamily Filarioidea. Nematode larvae of the other seven isolates, four from domestic pigs, one from a yellow-legged gull (*Larus michahellis*) and two from unknown hosts were not identified due to the DNA degradation caused probably by the digestion process. The recovery of nematodes different from those of the genus *Trichinella* during routine meat inspection, seems to be not so unusual showing the need to inform technicians in charge for the analysis about the possibility of false positives, and the need for a molecular based identification system allowing a reliable and quick response, i.e. a DNA barcoding like system.

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75 *Trichinella* spp. infections in different host species of an endemic district of Serbia

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Keywords: *Trichinella* spp., species, wild animals, zoonotic, infection

Trichinella spp. infections are endemic in the Balkan region of Europe. In spite of the fact that these zoonotic parasites are a serious problem for the human health and animal husbandry, only a limited number of isolates from Serbia have been identified at the species level so far. The aims of the present study were the surveillance and monitoring of *Trichinella* spp. in domestic pigs, and synanthropic and wild animals and to investigate the *Trichinella* species circulating in Serbia. During the 2009-2010 period, muscle samples were collected from 167 wild animals: 94 wild boars (*Sus scrofa*), 57 red foxes (*Vulpes vulpes*), 13 golden jackals (*Canis aureus*), 3 wolves (*Canis lupus*) and 116,398 domestic pigs. Muscle samples from wild animals were analyzed by artificial digestion and muscle samples from domestic pigs were analyzed by trichinoscopy and artificial digestion. Genotyping was performed by multiplex PCR. GIS was used for mapping the spatial distribution of animals infected with *Trichinella* spp.. *Trichinella* spp. infection was detected in 302 (0.26%) domestic pigs, in 11 (11.7%) wild boars, 7 (12.3%) red foxes, 7 (53.8%) golden jackal, and 3 (100%) wolves. *Trichinella spiralis* and *Trichinella britovi* were the only two species identified in the isolates, occurring either as single or mixed infections. The identification of *Trichinella* spp. positive animals allowed to identify the foci of transmission and to inform the veterinary services, the owners of pig farms and slaughterhouses and hunter's associations about the risk of transmission of these zoonotic agents. The results point out the circulating of *Trichinella* species by a domestic or a sylvatic cycle, the transmission between these two cycles, and the role of some host species as reservoirs of *T. spiralis* or *T. britovi* or of both species in Serbia.

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76 Cross-breeding between *Trichinella* T12 and the other encapsulated *Trichinella* species suggests its reproductive isolation

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Keywords: *Trichinella* T12, cross-breeding, reproductive isolation, Argentina, *Trichinella* genus

Trichinella T12 is a new genotype of the genus *Trichinella* recently discovered in cougars (*Puma concolor*) of Argentina. Larvae encapsulate in the muscle cells. It is the only autochthonous genotype discovered in South America so far. To deeply investigate the biological relationship of this taxon with the other encapsulated taxa of the genus *Trichinella*, breeding experiments were carried out in laboratory mice by the inoculation per os of one male or one female larva of *Trichinella* T12 and one male or a female larva of the other five encapsulated species recognised so far (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, and *T. nelsoni*). Mice were killed 40 days p.i., and the entire skinned and eviscerated carcasses were digested individually. To evaluate the fertility of the F1 hybrid muscle larvae produced by these experiments, single couples and 100 pooled larvae (both males and females) were crossed in mice. Single male and female adult worms of *Trichinella* T12 only crossed with female and male worms of *T. britovi* and *T. murrelli* in both directions. Only four hybrid larvae were collected from a mouse which had been infected with a *T. nativa* male larva and a *Trichinella* T12 female larva. Nevertheless, no larvae were recovered from mice infected with the F1 offspring (both single couples and pooled couples originating from *T. britovi* × *Trichinella* T12 and from *T. murrelli* × *Trichinella* T12, in both directions). The results of the cross-breeding experiments suggest that *Trichinella* T12 is reproductively more related to *T. britovi* and *T. murrelli* than to any of the other encapsulated species even if the infertility of the F1 larvae suggests the reproductive isolation of this taxon. Post zygotic isolation mechanisms are acting in such a way to prevent the gene flow between *Trichinella* T12 and *T. britovi* or *T. murrelli*. Long time barriers to gene flow produce an accumulation of independent mutations in different genetic lineages, increasing genetic distances among the different species.

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77 Inferring the history of *Trichinella* diversification and dissemination from analyses of genetic variability: Recent achievements, future potential, and practical limitations

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Keywords: *Trichinella*, epidemiology, evolution, phylogeny, microsatellites, diversity

Diagnosing species of *Trichinella* by genetic means has become an invaluable tool for assessing parasite epidemiology, but only rarely have such methods yet been used to study parasite diversification and dissemination history. As first reported at ICT12, *T. spiralis* harbors substantially less genetic variability in Europe than does *T. britovi,* and *T. spiralis* became established in North and South America by means of introduction from European. These inferences were derived from analysis of variation in maternally inherited mitochondrial genes, and from highly polymorphic microsatellite repeats encoded in the nuclear genome. Since then, such markers have been employed to investigate whether *T. spiralis* in East Asia is more genetically diverse than elsewhere, to investigate the extent and type of gene flow occurring between Arctic populations of *T. nativa* and the T6 genotype. Here, I present evidence in favor of the first hypothesis and describe equivocal evidence in the latter case, using each as an opportunity to illustrate the possibilities and limitations when using multi-locus genotyping to characterize *Trichinella* isolated from naturally infected hosts either as individual larvae or pools of larvae.

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78 Use of Nuclear Microsatellites in Genetic Variability Assessment of *Trichinella* Isolates

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Keywords: trichinellosis, microsatellites, genetic variability

Trichinellosis is a food-borne parasitic zoonosis with a yearly incidence of about 10,000 clinical cases worldwide. It is one of the most serious zoonotic diseases in Romania with more than 28,000 human cases reported over the last 25 years. *Trichinella* species and genotypes are present on all continents, from the tropical regions to cold ones. To date, there have been identified four *Trichinella* species in Europe: *T. spiralis, T. nativa, T. britovi* and *T. pseudospiralis.* In order to identify a genetic variability in ecologically distinct species of *Trichinella,* which may lead to a potential geographical disposition of *Trichinella* isolates, we tested the variability of nine nuclear microsatellite loci in a small population of *Trichinella* isolates of Romania. We obtained amplification for most of the microsatellites for isolates of both *Trichinella spiralis* and *T. britovi*. Size-differences were evident between the amplicons generated by from *T. spiralis* vs *T. britovi,* and also among three isolates of *T. spiralis* (designated 7.97, 7.42, 7.29) at 2 of the 9 microsatellites (TP43, TP32). For a more detailed analysis of the differences obtained in this study, we plan to use the fluorescently (6-FAM) marked primers, for sequencing and genotyping of the amplification products.

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79 Travel: a New Driver for Trichinellosis

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Keywords: *Trichinella*, epidemiology, travel

Acquiring trichinellosis while travelling abroad is quite a frequent occurrence, but most of the time sporadic cases are hidden by high incidence of autochthonous cases. It is not a new phenomenon, as McAuley et al (1991) reviewing "*Trichinella* infection in travellers" in the US from 1975 to 1989 reported 26 cases after pork consumption while travelling in Central America. In France, since 1975, 67 cases were contracted abroad, while 2497 cases were contracted in the country and related to 8 outbreaks due to horsemeat consumption (each, involving from 7 to 642 cases). However, implementation of radical preventive measures (education of technicians, quality control, and lab accreditation) has prevented the occurrence of new horsemeat related outbreaks since 1998. Since then, 28 imported cases represent 37% of all cases reported to the NRC, with a mean annual incidence of 2 cases. Between 1975 and 1998, 40 imported cases represented only 1.5% of all identified cases but with a comparable mean annual incidence of 1.6 cases (Ancelle et al. 2005, NRC report 2006-2010 http://www.univ-paris5.fr/cgi-bin/WebObjects/WODownload?no=83905). Incidence of imported cases could even have decreased as numbers of international travellers increased during that period. In cases diagnosed in France, most cases were acquired in Egypt, Turkey, Algeria in the period 75-95 (source: pork or wild boar); since 1995, most cases were acquired in Laos, West Africa and Canada (source: pork, warthog, bear). During the last decade, occasional cases were imported in European countries from East European countries (Poland, Romania, former Yugoslavia) where trichinellosis re-emerged after social upheavals of the 90s (Angheben et al, 2008; Nöckler et al., 2007; Milne et al, 2001). Cases were also imported in Asia after turtle consumption (Maeda & Kawana, 2011) and after travelling to a sea-side resort in a neighbouring island of Singapore (Kurup et al., 2000).

Imported cases are most likely to occur in developed countries, but are certainly good indicators of the epidemiology of the disease and may reveal a high transmission in some countries where the disease is or had become unknown (e.g. Senegal, Laos…). In addition, consumers from countries where the habit of eating raw meat is common will certainly be at higher risk and particularly if they are backpackers, adventure travellers or hunters of exotic animals. Therefore, travellers should be informed about the risks of eating raw meat (pork and pork products, game or reptile meat) and should be discouraged from illegally importing potentially infected meat which could introduce the parasite in *Trichinella*-free areas.

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80 The Worldwide Occurrence and Impact of Human Trichinellosis, 1986-2009

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***Keywords*:** trichinellosis, epidemiology, prevalence, incidence, deaths

To assess the global incidence and clinical impacts of human trichinellosis, outbreak reports and single case data were analyzed for 1986-2009. Searches of six international databases yielded 494 reports. After applying strict criteria for relevance and reliability, 261 reports were selected for data extraction. In the period under study, there were 65,818 cases and 42 deaths reported from 41 countries. The Euro region accounted for 86.5% of cases, of which 50.2% occurred in Romania (mainly during the 1990-99 period). Incidence rates in the endemic countries ranged from 1.1 to 16.1. From 1986 to 2009, autochthonous cases of trichinellosis were documented only in: Ethiopia for the AFRO region; Argentina, Canada, Chile and Mexico for the AMRO region; Lebanon and Iran for the EMRO region; 29 countries of the EURO region; India and Thailand for the SEARO region; and China, Korea, Laos, Singapore and Vietnam for the WPRO region. Trichinellosis infections acquired in countries different from those where the disease was developed and diagnosed, were documented in 8 European countries, China and Japan. Clinical reports demonstrated trichinellosis affects primarily adults, about equally in males (51%) and females, with a median age of 33.1 years. Clinical effects, based on 5,377 well-described cases, were myalgia, diarrhea, fever, facial edema, and headaches, which after treatment disappeared within 2-8 weeks. Pork was the major source of infection, although wild game sources (mainly wild boar) were important. Other sources of infection were horse meat, dog meat and turtle meat. The paucity of data on long-term clinical follow-up does not allow addressing the issue of chronic trichinellosis. These data will be valuable to calculation of morbidity burden estimates (DALYs).

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81 Clinical Manifestations in Human Trichinellosis-Retrospective Epidemiological Study

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Keywords: myalgia, edema, fever, other clinical signs

The presence of *Trichinella* parasites in humans does not always manifest overt clinical symptoms, the clinical manifestations in trichinellosis being polymorphic and posing trouble for the clinician. To advance certainty in the diagnosis of the disease, we conducted a retrospective analysis of 143 cases of human trichinellosis hospitalized during 2001-2008 in the Infectious Diseases Hospital from Brasov. The study's data derived from observation sheets from the hospital archives of all existing cases in the file, of which 124 cases were admitted to the Adults Ward and 19 cases in Children Ward. Of the total cases admitted, the erroneous diagnoses at admission totaled 11%, of which 4% were diagnosed with febrile syndrome of unknown etiology, 2% - acute enterocolitis and 5% - other diagnoses (respiratory virus infection, acute upper respiratory tract infection, acute sinusitis, sepsis of unknown etiology, eosinophilia of unknown etiology, echinococcosis, acute hepatitis).

Most adults manifested moderate symptoms (64%); asymptomatic and mild cases amenable to ambulatory treatment amounted to 31% of the total, severe cases represented 5% of the total. Clinical presentation in children showed similar proportions for mild (16%) and severe (5%) forms; cases were most frequently asymptomatic (42%), were moderately symptomatic 37% of the time. To aid future diagnosis of trichinellosis, three elements (myalgia, edema, fever) are followed. Analysing in this manner the patients group, we found that these symptoms are present at the majority of cases, leading to an accurate diagnosis in 89%. In terms of gastrointestinal manifestations, the results obtained from the whole group show that 46% of patients had experienced nausea, vomiting, and/or diarrhea syndrome before or during the hospitalization. Only 34% experienced such symptoms. Of the 143 hospitalized patients, 30 subjects experienced other types of clinical manifestations: cardiac (30%), dermatologic (20%), hepatic cytolysis 13%, respiratory and allergy (10% each), neurological and ganglionic (each 7%) and urinary symptoms (3%). Thus, attention to the triad of myalgia, edema, and fever promotes diagnosis of human trichinellosis.

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82 Seroprevalence of *Trichinella* antibodies in blood donors in France

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Keywords: trichinellosis, *Trichinella*, Western blot, cross reaction, *Capillaria*, *Trichuris*, blood donors

Though 2565 cases of trichinellosis have been reported in France between 1975 and 2011, during 32 outbreaks related to horse, wild boar or bear meat, France belongs to the countries with a low risk of infection. Moreover, some regions of France are considered to be free from human cases. In order to better understand the epidemiology of this parasitosis in humans, we carried out an investigation on the seroprevalence of trichinellosis in the "Two-Sèvres" department where no outbreak or cases have been reported so far. We used a new Western-blot (WB) kit developed by LDBIO Diagnostics using excreted-secreted antigens. Amongst the 500 blood donors tested, 44 (8.8%) showed characteristic profiles of anti-*Trichinella* antibodies. No significant difference of the prevalence was associated with gender, blood group or age. These results were particularly amazing since no case of trichinellosis has been reported in this district either in humans or domestic or wild animals. A 22 % prevalence was observed, using the same WB kit, in 147 blood donors from Algeria (Zait & Hamrioui, pers. com.), a country where pork is not consumed. These *Trichinella* positive blood donors were seronegative for hydatidosis, echinococcosis, and cysticercosis. Different batches of sera coming from patients with various parasitosis were tested with the new kit and exhibited seroprevalences ranging from 3.3% to 45.5 %. These results could be linked to a surprising lack of specificity of this test based on ES antigens but could also suggest either an infra-clinical infection caused by the ingestion of very low doses of *Trichinella* larvae present in meats or cross-reactions with related parasites such as *Trichuris vulpis* from dogs or *Capillaria hepatica* from rodents and contaminating food.

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83 Description of an outbreak of human Trichinellosis in an area of Argentina historically free of infection: Importance of surveillance studies

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Keywords: human trichinellosis, outbreak, *Trichinella*-free area, Argentina, diagnosis, lack of surveillance.

Recent studies have demonstrated the presence of *Trichinella spiralis* infection in areas previously free of infection. However, an outbreak of human trichinellosis occurred in that area from Argentina (Gualeguaychú, Entre Ríos) in July 2010. A total of 62 individuals were infected by pork products procured from a commercial source. The number of individuals positive for trichinellosis was 56 (90%); 44/56 were confirmed by laboratory testing (71%) and 12/56 (19%) by epidemiologic nexus. Clinical characteristics, laboratory parameters and inmunoserological (IS) specific testing (ELISA, IIF and/or immunoelectrotransference) of the individuals involved were evaluated. Food samples were analyzed by artificial digestion. The results showed: a) Parasitic burden in food: 1.1 ML/g, being *T. spiralis* the species found. b) Affected individuals' mean age: 39 years old. Age-adjusted rate (years) of confirmed cases: 0-14: 9% (n=5); 15-29: 18% (n=10); 30-49: 52% (n=29); 50-64: 19% (n=11) and >65: 2% (n=1); by genre: 66% (n=37) men and 34% (n=19) women. c) Most frequent symptoms: myalgia (93%, n=50), bipalpebral oedema (78%, n=42) and fever (70%, n=38). The 26 patients confirmed (46%) received anti-parasitic and anti-inflammatory treatment, and 7 (12%) individuals anti-inflammatory treatment. Patients evolved satisfactorily without side effects. Hospitalization was not necessary. d) Patients studied for eosinophils count (n=34) presented increased values (mean value, mv: 25.3%). Mean leukocyte count: 11047/mm^3. e) Infection was confirmed one month post-ingestion in 73% (n=33) of the 44 individuals diagnosed. f) CPK and LDH values higher than normal values were detected in 15/24 (62%) and in 9/20 (45%) serum with mvCPK: 687 UI/l and mvLDH: 583 UI/l. g) There are no control centers for pork infection detection in the area. Lack of sanitary controls of pork, and the lack of knowledge regarding this infection, led to this outbreak in an area historically free of this parasitosis. This lack of local control socially and economically affected all the pork production chain (farmers, traders, prime age individuals). Previous studies in the area helped human and animal health professionals to conduct immediate prevention and control measures.

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84 Bear Meat related trichinellosis: an emerging zoonosis amongst French tourists in the Canadian Arctic

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Keywords: bear, trichinellosis, *Trichinella*, human infection

Five cases of trichinellosis with onset of symptoms in September 2009, were reported in France and were linked to the consumption of meat from a grizzly bear (*Ursus arctos*) in Cambridge Bay in Nunavut, Canada where this crew stopped while sailing the NW passage (Houzé et al. 2009). Three additional cases where reported in another crew who also ate parts of the same bear. Including the present report, a total of 25 cases linked to bear meat consumption has been reported to the French NRC since 1995: 2 cases in 1995 from polar bear (*U. maritimus*) in Greenland, 1 case in 2004 & 17 cases in 2005 from black-bear (*U. americanus*) meat from northern Quebec, Canada (Ancelle et al., 2005). Trichinellosis is a widespread helminthic zoonosis endemic in northern Canada where walrus (*Odobenus rosmarus*) meat is the most frequent source of trichinellosis infection in humans. Gajadhar and Forbes (2009) reported a 29.4% prevalence of *Trichinella* in Canadian grizzly bears (65.9% for polar bears, 40.6% for walrus & 7.3% for black bears). Outbreaks have been described in Nunavut on Baffin Island and Repulse Bay after consumption of walrus meat. Inuit populations consume bear meat thoroughly cooked whereas walrus meat is eaten frozen, fermented or air-dried (Forbes et al., 2003). Other outbreaks have been described in neighbouring Nunavik (from Inukjuak on south Hudson Bay and as far north as Salluit) leading to a prevention program in Inuit communities (see Houzé et al., 2009). The arctic species of *Trichinella* (*T. nativa* and T6) are resistant to freezing and are killed by sufficiently cooking at 67°C. Travellers should be aware of the risks of eating game meat such as bear or walrus meat. French tourists are particularly at risk because of the well-known French habit of eating meat raw or rare.

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85 A 10 Year Retrospective Epidemiologic Survey of the Diagnostic Errors and their Implications in Human Trichinellosis Development in Brasov County, Romania

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Keywords: diagnostic errors, epidemiological correlation

Certainty diagnosis has frequent difficulties related to the limit of the methods, and is the reason to promote complex diagnosis, which corroborates the results of clinical, laboratory and epidemiological, epizootiological investigations. Objectives and Methods Analysis of diagnostic errors in human trichinellosis and their implications increase hospitalization, treatments costs, medium-severe and severe clinical evolutions, and chronic disease. Over 10 years, a retrospective analytic survey of patients in the Infectious Diseases Hospital from Brasov finally confirmed 699 cases of trichinellosis. Data sources were obtained from Public Health Authority of Brasov County and the Infectious Diseases Hospital in Brasov. Of all 699 trichinellosis cases, 314 (44.92%) were initially diagnosed incorrectly. Depending on the first diagnosis patients were refered to the hospital, and trichinellosis was confused with other 26 diseases. Digestive manifestations rank first among diagnostic errors (41.7%). Trichinellosis passes at the onset through a series of clinical signs and symptoms of digestive sphere, followed by respiratory pathology errors (32.49%). In blood dissemination phase of the disease, there were erroneous diagnoses of allergic (4.40%), renal (4.10%), eye (2.80%) and even neuropsychological diseases (4.40%), which removed the patient from starting an effective antiparasitic treatment. The average time between the date of getting ill and the date of trichinellosis detection changed from 16.42 to 5.41 days because of the immediate establishment of etiologic, pathogenic and symptomatic treatments. Early detection of disease has beneficial repercussions on the average duration of hospitalization; it decreased from 41.25 to 9.84 days. Diagnostic errors are found in varying percentages: in childhood – 20%), in old age – 36%, and for the active age – 50%. For women, the symptomatology is polymorphic, and the rule is usually an incorrect diagnosis (56%) *vs.* for males – 36%. In rural areas, diagnostic errors are more common (59%) *vs.* the urban areas (42%). In medium forms of disease, the diagnostic error curve follows the correct diagnosis curve, the clinician changes lately the wrong diagnosis; among these trichinellosis forms there are the future

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patients with chronic trichinellosis. In conclusion diagnostic errors in human trichinellosis in Brasov County, Romania are due to clinical manifestations polymorphism. They involve the increase in the duration and direct and indirect costs of hospitalization.

86 Eosinophils regulate local immunity during *Trichinella spiralis* infection

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Keywords: Eosinophils, immunity, *Trichinella*

Establishment of chronic infection is an important feature of many parasitic diseases, yet the immune regulatory mechanisms that support the long-term survival of parasites in infected hosts remain poorly understood. *Trichinella spiralis* initiates the chronic phase of infection when newborn, first-stage larvae infect skeletal muscle cells. We have discovered that eosinophils contribute to the ensuing myositis while simultaneously protecting larvae against immune-mediated destruction. Specifically, we find that growing larvae are killed in large numbers in Phil and dblGATA mice and, furthermore, that parasite survival improves when eosinophils are restored to such mice. Thus, the long-standing paradigm of eosinophil toxicity in nematode infection requires reevaluation, as eosinophils appear to regulate the immune response in a manner that supports chronic infection and insures worm survival in the host population. Our goal is to elucidate the properties and actions of eosinophils that enable them to sustain infection. We have found that eosinophils promote local recruitment of lymphocytes and activation of macrophages that protect parasites during a vulnerable phase of growth.

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87 "Evaluation of different immunization protocols to induce protective immunity to *Trichinella spiralis* using TSL1 antigens in a murine experimental model"

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Trichinellosis is a public health hazard and an economic problem in porcine animal production and food safety for humans. Therefore, the development of methods for its prevention and control are most relevant. Protection against *T spiralis* in experimental models has been achieved using a variety of muscle larvae (ML) antigens such as TSL-1 antigens (TSL-1 Ag), recombinant proteins or peptides expressed in live vectors using different adjuvants and DNA immunization. Our group has designed various immunization protocols which include the use of: a 30-mer peptide from *T. spiralis* 43 kDa antigen expressed in a live vector (*Salmonella* enteric serovar *typhimurium*), the protein Lumazine synthase (LS) of *Brucella* sp. and the Transfer Factor (TF) obtained from crocodile, together with TLS-1 antigens to immunize BALB/c mice previous to the challenge infection with *T. spiralis* ML. In general, the results showed an approximately 60% reduction in ML burdens in the immunized animal with slight variations according to the protocols used. In the case on animals immunized with the peptide expressed in the live vector or with TSL-1 antigens and Lumazine synthase, a 45-70 % reduction in adult worms was observed. Cytokine profiles determined in the intestinal fluids of immunized mice with TLS-1 antigens and LS or TF showed an early induction of Th1 type cytokines followed by a transitory increase of Th2 type cytokines. In animals immunized with TLS-1 antigens and LS, an early increased expression of activated macrophages markers (Fizz 1 and Arg1), and a decreased of Tregs cells in the mesenteric lymph nodes, was observed. All together, these results suggest that the use of the 30-mer peptide expressed in *S. typhimurium* or TSL-1 Ag with different immunization protocols induced a significant protection against *T. spiralis*. Therefore, these protocols may be used with other *T. spiralis* antigens to potentiate their capacity to induce protective responses in the host against this parasite.

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88 Effects of *Helicobacter pylori* neutrophil-activating protein on the protective role of IgE, eosinophils and on myositis in experimental trichinellosis

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Keywords: Experimental trichinellosis, mouse, Th1 adjuvant, *Helicobacter pylori* neutrophil activating protein

Trichinella spiralis infection elicits a Th2 immune response, characterized by high levels of IgE and eosinophils. Using recombinant *Helicobacter pylori* neutrophil activating protein (HP-NAP), a Th1 adjuvant, we evaluated the modulating effects on experimental *T. spiralis* infection at muscle level. BALB/c female mice were orally infected with 350 *T. spiralis* muscle larvae (ML) and divided in two groups: G1) treated with intraperitoneal (i.p.) PBS; G2) treated with 10 μg of HP-NAP i.p. on days 10 and 28 post infection (d.p.i.). At 42 d.p.i. mice were sacrificed and the tongue was collected from each animal and processed for routine histology. Tongue sections were stained with H&E for subsequent image acquisition and analysis. The number of eosinophils present in the inflammatory infiltrate around the nurse cell-parasite complex (NC-P) was counted on several sections of both group animals after Congo Red staining. Levels of matrix metalloprotease-9 (MMP-9) were evaluated in the plasma of different animal groups at 42 d.p.i., by zimography. The skinned carcasses of each mouse was eviscerated and digested in 1% HCl and pepsin to assess the parasite burden. Differences between the groups were determined using Student's *t* test, considering p values < 0.05 significant. The results showed that the ML burden was lower ($p < 0.02$) in G1 than in G2 animals. In both groups there was an inverse relationship between ML burden of each animal and total IgE level (G1: r -0.617, $p = 0.0013$ and G2: r -0.678, $p = 0.0001$) or blood eosinophil count, evaluated in the same mouse on day 42 (r -0.390, $p = 0.0592$ and r -0.803, $p = 0.0001$, respectively). Inflammatory response around the NC-P was significantly higher in G2 than in G1, but the number of eosinophils, counted around each complex, was significantly lower in G2. Preliminary results show that in Group 2 animals MMP-9

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active form (82 kDa) levels, as well as those of 125 kDa corresponding to Neutrophil-gelatinase associated lipocalin, secreted by neutrophils, were significantly higher than in Group 1 mice. Conversely, no difference was observed between these animal groups as regards MMP-9 pro-enzyme (92 kDa) levels. This study provides evidences for an anti-Th2 activity *in vivo* by HP-NAP and for the partial protective effect of Th2 responses in *T. spiralis* infection. Furthermore, HP-NAP resulted to exacerbate the myositis.

89 Exposure of pigs to encapsulated sylvatic isolates of *Trichinella* protects against a challenge infection with *T. spiralis*.

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Keywords: epidemiology, immunology, heterologous infection, swine, pork, *Trichinella*

The sylvatic genotypes of *Trichinella* occurring in North America have moderate to low infectivity for pigs as opposed to the domestic genotype, *T. spiralis,* which is very infective to pigs. Conversely, even though *T. spiralis* reproduces well in wild carnivore hosts, it is found in the U.S. almost exclusively in pigs and only rarely in wildlife species, where *T. murrelli* is the most prevalent genotype. The reason that *T. spiralis* has not spread to the U.S. wildlife carnivore population is not known, but it has been found that prior infection with *T. nativa* renders pigs refractory to infection with *T. spiralis* (Smith, 1986); the development of resistance in pigs to *T. spiralis* after exposure to other genotypes occurring in North America has not been studied. In the present study, pigs were given 10K muscle larvae (ML) each of *T. nativa, T. pseudospiralis*, *T. murrelli*, or *Trichinella*-T6 to induce a primary infection. After 60 days, pigs were challenged with 10K ML of *T. spiralis*, serum was collected biweekly from all pigs, and muscles from the diaphragm, tongue, masseters, and neck were collected at necropsy 45 days after challenge. Muscle samples were HCl-pepsin digested to determine worm burden per gram in each tissue. Species composition of collected larvae was determined by genotyping, comparing the size of PCR amplicons of the internal transcribed spacers and expansion segment V (ESV) of the rDNA repeat. Results indicate that primary infection of pigs with encapsulated genotypes of *Trichinella* (*T. nativa, T. murrelli*, or *Trichinella*-T6) circulating in the sylvatic ecosystem in North America renders pigs refractory to a challenge infection with *T. spiralis*, while a primary infection with a non-encapsulated genotype (*T. pseudospiralis*) provides no such protection against a challenge infection. This information is critical for a complete understanding of the true risk posed by sylvatic *Trichinella* genotypes in the environment to the growing population of pasture-raised pigs that have access to wildlife and wildlife carcasses potentially infected with these genotypes, and may provide information on generation of immunity to *Trichinella* in pigs which could lead to immunologically based strategies for protecting pasture-raised pigs from infection.

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90 "Let's seal the deal and fight autoimmunity" says *Trichinella* to dendrite cells.

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Keywords: *Trichinella*, immunological tolerance, dendritic cells, autoimmunity

Dendritic cells (DCs) are potent stimulators of adaptive immunity, but they are also crucial to ensure immunological peace. Namely, there is mounting evidence that DCs establish and maintain immunological tolerance by several mechanisms, including differentiation of regulatory T cells (Tregs), which are capable to contain autoimmunity and chronic inflammation. It has been shown that infection or products from some helminth pathogens promote DC tolerogenicity and induce differentiation of Tregs. This way, parasite persistence is favored and infection-induced pathology is limited. At the same time, immune responses to other antigens are modulated. *In vivo* experiments previously showed that *Trichinella spiralis* infection increases the number of Tregs at the place of muscle infection and in the spleen of infected rodents. However, whether and how *Trichinella*-derived products act on DCs to induce Tregs has not been determined. Our previous results indicated that antigens from all *T. spiralis* life stages are capable to induce tolerogenic status of DCs *in vitro*. Here we emphasize the role of DCs educated by excretory-secretory products (ES) as potent promoters of naive T cell differentiation and polarization towards mixed Th1/Th2 response with Tregs involvement *in vivo* but not *in vitro*. Application of those DCs to DA rats exposed to induction of experimental autoimmune encephalomyelitis, indicated that the disease could be successfully ameliorated in time and dose dependent manner by DCs injection. This finding could support therapeutic potential of *Trichinella*-educated DCs for treating a variety of immune disorders.

91 Vaccination against *Trichinella spiralis* in pigs: a high protection induced by a combination of recombinant proteins

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Development of vaccines to protect pigs against *Trichinella* could be a prophylactic method to prevent human trichinellosis. Indeed, our laboratory has set up a strategy to target antigens expressed at early stage of parasite development and that might be involved in the host invasion. Vaccination was carried on pathogen-free as well as conventional pigs following a protocol previously validated in mice (Deville *et al.,* 2005). Briefly, a first intra-muscular injection was performed using recombinant proteins associated with a water in oil in water adjuvant Montanide™ ISA206VG. A boost was achieved at 28 days post-immunization (dpi) in the same conditions. The challenge infection occurred at 56 dpi with 1000 muscle larvae (ML) of *T. spiralis* (ISS 004) inoculated *per os*. Then protection of animals was assessed by ML burden evaluation in muscles (tongue, masseters, diaphragm pillars, brachial triceps and femoral biceps) at 90 dpi. Counting of ML was performed after artificial digestion of individual muscles and expressed by number of larvae/gram of muscle. Pigs were immunized with recombinant proteins such as NBL1 (serine protease specific of newborn larvae stage), NBL2 (cystatin protein), TsGST24 (*T. spiralis* glutathione S transferase 24 kDa) and 411 (immunogenic peptide). The association of these four proteins induced a global reduction of 95% of ML burden compared to control animals (pigs receiving 1000 ML without vaccination). This protection was associated with the production of IgG1 and IgG2 directed against the recombinant proteins as well as the synthesis of IL5 and IL6. When NBL1 was discarded from the protein cocktail for immunization, the observed protection level dropped to 44.94%. Finally, vaccination of pigs with NBL1 alone induced 72.19% of ML burden reduction. This work describes for the first time the efficiency of an anti-*T. spiralis* vaccine, which is based on the use of recombinant proteins and a safe adjuvant. The role of NBL1 in the observed protection is major since by itself it strongly inhibited the installation of the parasite in muscles. This result confirms thus the role of NBL1 in invasion of the host by *Trichinella*. Although the parasite installation within its host is not totally blocked yet, the potential to increase the level of protection may improve by selection of new targets expressed either at early stage of *Trichinella* development and/or exhibiting a key function for the parasite survival.

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92 Some aspects of humoral and cellular immune responses in swine experimental *Trichinella spiralis* infection

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Keywords: humoral immunity, cellular immunity, lysozyme synthesis, *Trichinella*, pigs

The pathogenicity of *Trichinella spiralis,* in humans, is higher than that of other species because of a higher number of newborn larvae produced by the females and a stronger immune reaction induced, relative to other genotypes. A total of 13 Large White pigs, aged 2 months, were divided in two groups, 6 were infected with 1500 *Trichinella spiralis* larvae per kg bodyweight and 7 were used as controls. During the first 8 weeks post infection, cellular and humoral immune response were evaluated by *in vitro* phagocytosis assay, phytohemagglutinin (PHA)-stimulated blastic transformation test, lysozyme activity evaluation and enzyme-linked immunosorbent assay (ELISA). A slight increase of the phagocytic activity was registered 14 days p.i. in the infected group, but the differences between the infected and control groups were not statistically significant ($p>0.05$). Significantly increased lymphocyte activity was observed 30 ($p=0.003$) and 60 days ($p=0.05$) p.i., in *Trichinella* infected pigs comparing with uninfected ones. Moderated increased serum lysozyme levels (61.2 µg/ml) were registered during the intestinal phase of infection while lysozyme synthesis decreased during the parasite migration and returned normal in the muscular phase. The specific IgG antibodies were detectable by ELISA at 30 days p.i. only in infected group. In conclusion, the innate and the adaptive immune system response, during the *Trichinella spiralis* infection in pigs, is moderated suggesting a certain equilibrium between host and parasite.

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93 Effect on apoptosis associated protein expression of H7402 cells co-incubated with polypeptide protein of *Trichinella spiralis*

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Keywords: *Trichinella spiralis*, p53, bcl-2, survivin, cancer, tumor suppression

Antitumor effects of *Trichinella spiralis* have been reported from infection, which is interesting owing to similarities between the *T. spiralis* encystment mechanism and the signal regulation mechanism of tumor cell apoptosis. However, the active antitumor ingredient of *T. spiralis* remains unknown. Human hepatoma carcinoma cells (H7402) were treated with 0.140 mg/ml polypide protein, and then analyzed for expression of p53, bcl-2 and survivin by immunohistochemistry, flow cytometry and confocal microscopy. The results showed that p53 expression in treated H7402 cells was significantly upregulated compared with untreated H7402 cells. Bcl-2 and survivin expression in treated H7402 cells was significantly downregulated compared with untreated H7402 cells ($P<0.05$). We speculate that *T. spiralis* polypide protein could contain antitumor ingredients and effectively regulate apoptosis-related genes and trigger a mitochondrial pathway or death receptor pathway that could lead to tumor or cancer cell apoptosis. The antitumor ingredient of *T. spiralis* could contribute to targeted human hepatoma carcinoma therapy by regulating apoptosis-related genes.

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94 Antigen-specific T cell immune response by co-immunization with the Ts87 DNA vaccine and recombinant Ts87

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Keywords: cytokines, *Trichinella*, autoimmune disease inflammation, Th1, Th2, Treg

Epidemiological studies suggest that inflammatory bowel disease (IBD) is rare in countries where intestinal nematode infections are common. T cells constitute an important part of many immune responses, including those associated with IBD and intestinal nematode infection. Some researchers reported that the simultaneous co-immunization with a DNA vaccine and its cognate coded protein antigen could induce antigen-specific Treg cells, demonstrating its potential to protect animals from autoimmune disease. Previously we reported that oral immunization of mice with Ts87 DNA delivered in *S. typhimurium* elicited immunosuppressive cytokine IL-10 secreted by the Treg cells. In this report, female C57BL/6 mice were divided into three groups. They were immunized with plasmid DNA at 100μg, protein at 100μg, or a combination of both at 100μg each as the vaccine regimens, respectively, into tibialis anterior muscle on days 0, 14 and 28. Antibody responses showed that anti-Ts87 IgG levels and total IgE levels in co-immunization group were greatly increased following the second and third immunizations. Mouse subclass IgG responses showed that three groups induced a relatively balanced IgG2a/IgG1 (Th1/Th2) response. ELISPOT assay was used to detect cytokines of Th1 (IFN-γ, IL-2), Th2 (IL-4, IL-5, IL-6), Treg (IL-10), and Th17 (IL-17) secreted by splenocytes of mice. Cytokine profiling showed a significant increase in IFN-γ, IL-2, IL-4, IL-6, IL-10 in splenocytes of co-immunization mice. But the expression of IL-5 and IL-17 was down-regulated in the co-immunization group significantly. Therefore, Co-immunization with recombinant Ts87 and pvax1-Ts87 can induce the humoral immune response and increase expression of regulatory cytokines (IL-10) while suppressing expression of proinflammatory cytokines (IL-17).

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95 *In vitro* analysis of cellular activation of mouse mast cells stimulated by *Trichinella* antigens

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Keywords: *Trichinella spiralis*, BMMC, T cells, dendritic cells, activation, cytokines, chemokines, costimulatory molecules

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During *Trichinella* infection, the intestinal epithelium of the host is the first tissue invaded by this parasite nematode. Immune cells of the gut associated lymphoid tissue (GALT) develop an innate response followed by a specific immune response leading to a protection against a reinfection, but failed to expulse the parasite. This response, described as Th2 type, depends on T $CD4^+$ lymphocytes. In GALT, mast cells (MCs) play an essential role in innate immunity and are considered as sentinels of mucosal immune systems. These cells are also described as a link between innate and specific immunity. It has been recently reported that MCs could be involved in specific immunity, due to the cytokines they release. Many studies underline the great part led by MCs during infection by *T. spiralis* at intestinal stage with a focus on innate immune response. However, the cellular mechanisms leading to the Th2 protective immunity are still not understood. Indeed, the cellular cooperations involved in the link between innate and specific immunity during trichinellosis are not characterized. The aim of this study is to highlight the role of MCs as an initiator of Th2 profile activation in the immune response against *T. spiralis*. MCs were obtained from mice bone marrow (BMMC), and then cultured with appropriate growth factors. These BMMC were activated either by total crude antigens from different parasitic stages or by recombinant antigens produced in our lab. Among these antigens, the immunogeneic peptide 411 was able to stimulate the release of tumor necrosis factor (TNF) and histamine. Microarray analysis of these stimulated BMMC demonstrated that cells are induced to express surface or soluble molecules directly involved in communication with T lymphocytes. Indeed, an up regulation of costimulatory molecules mRNA such as CD40, CD69, CD70, CD74 and class I/II major histocompatibility complex (MHC), cytokines such as IL-1α, IL-1β, and chemokines such as CXCL1 and CCL5 participate in T lymphocyte

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activation. Moreover, like dendritic cells (DCs), analysis by flow cytometry of OVA-DQ showed that BMMC were able to process this antigen in a dose and time dependant mechanism. Taken together, these results are in favour of a direct role of BMMC as antigen-presenting cells to T lymphocytes during the intestinal stage of *Trichinella* infection with an ability to process antigens, to express MHC molecules and costimulatory molecules as well as cytokines and chemokines interacting with T cells.

96 Lung cells involved in the immune defense mechanism in *Trichinella spiralis* infection

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Keywords: lung cells, helminthocytotoxicity antibodies dependent, newborn larvae, specific immunoglobulin isotypes

The function of leukocytes as effector cells against newborn larvae (NBL) in the presence of specific antibodies (Abs) by the Abs-Dependent Cell Cytotoxicity (ADCC) mechanism is well known. Considering the lung as a site for larvae retention and destruction, our aim was to evaluate whether leukocytes from lung parenchyma acted as effectors in the NBL death through an experimental infection using rats. We developed an *in vitro* ADCC where NBL were in contact with lung cells obtained from infected Wistar rats at day 6 p.i. or from non-infected cells, and anti-NBL surface cytotoxic serum (CS) or normal rat serum (NRS) as positive and negative controls respectively. Furthermore, the presence of anti-NBL surface isotypes, titer (n=7, IIF) and cytotoxic capacity from rat sera at day 6 and 13 p.i. (IIF anti-NBL surface positive) were studied. The relationship cell-number/NBL *vs.* mortality % was compared among cells from infected (day 6 p.i.) and non-infected rats. These methodologies were conducted at day 6 p.i. because NBLs are shed into the bloodstream and lymphatic circulation from the gut by about day 5 p.i. The results showed that: 1- cells from non-infected rats were able to kill NBL in the presence of Abs (% NBL mortality in the presence of CS 34.9±3.3 *vs.* 7.1± 2.7 NRS). 2- Cells from infected animals (day 6 p.i.) showed higher cytotoxic activity (p=0.0254) in the presence of CS than those from non-infected rats (% NBL mortality 57.9±8.2 *vs.* 34.9±3.3). 3- NBL mortality depended on ADCC assay of the relationship cell-number/NBL, obtaining higher mortality when cells from infected rats were used. 4- Isotypes anti-NBL surface were detected in rat's sera at days 6 and 13 p.i.: IgE and IgG2a in 63% and 13% of the animals, respectively; at day 13 p.i. all isotypes studied were detected: IgE was found in 100%; IgG2a and IgG1 in 86% and IgA in 71% of the animals respectively. 5- Abs present in rats sera at day 13 p.i. showed the property to mediate ADCC, presented a significant (p=0.0145) high mortality against NBL in comparison with NRS (% NBL mortality: 17.0±3.5 *vs.* 7.0±1.6 respectively). This coincided with the higher Abs titers found at this day. These results suggest that lung cells are able to act in the NBL death mechanism in the presence of effector serum Abs and that heminthocytotoxicity increases with cells from infected animals.

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97 Invasion of mouse primary enterocytes *in vitro* by *Trichinella spiralis* infective larvae

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Keywords: *Trichinella*, invasion, intestinal epithelium, culture

It has been known for many years that *Trichinella spiralis* initiates infection by penetrating the columnar epithelium of the small intestine; however, the mechanisms used by the parasite in the establishment of its intramulticellular niche in the intestine are unknown. Although previous observations indicated that the invasion also occurs *in vitro* when *T. spiralis* infective larvae are inoculated onto cultures of intestinal epithelial cells (e.g., human colonic carcinoma cell line Caco-2, HCT-8), a normal readily manipulated *in vitro* model has not been established because of difficulties in the culture of primary intestinal epithelial cells. In this study, we described a normal intestinal epithelium model in which the *T. spiralis* infective larvae were shown to invade primary intestinal epithelial cell monolayers *in vitro*. The larvae penetrated cells and migrated through them, leaving trails of dead cells in their wake. The cells derived from intestinal crypts of fetal mouse small intestine had the ability to proliferate continuously and to express specific cytokeratins as well as intestinal functional cell markers. Furthermore, primary normal intestinal epithelial cells were susceptible to invasion of *T. spiralis*, and those cells invaded by this parasite were heavily loaded with *T. spiralis* larval excretory-secretory antigens which were recognized by mouse immune sera. The normal intestinal epithelium model of invasion mimicking the natural environment *in vivo* provides a readily manipulated and controlled system to further investigate the process as well as the mechanisms by which *T. spiralis* establishes its intestinal niche.

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98 *Trichinella* as surgeons or influence of "Britov's vaccine" on the wounding process

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Keywords: *Trichinella*, "Britov's vaccine", cellular immunity, wounding process, regeneration

It is well established that immune status determines the seriousness of posttraumatic, infectious and wounding processes. Taking into consideration the importance of immunologic reactivity in the biology of wounding process, we sought to study the period of healing of experimental wholeskin wounds and the dynamics of wounding process in mice whose cellular immunity was induced by *trichinella* antigens using the method of V.A. Britov ("Britov's vaccine") and also in mice immunosuppressed by administering prednisolone. Four groups of mice were created: the first-controlled **(**intact mice); the second-immune mice (vaccinated with biopreparation from *Trichinella*); the third-immune mice administered prednisolone after vaccination; and the fourth group administered only prednisolone (modelling the condition of immunodeficiency). The complete epithelization of the skin defect of immunized animals in group 2 was over on the 19th day at the average meanings of 16.9 ± 0.4 days. In the control group, this process was complete on the 31st day (24.4 ± 1.8), in group 3 (vaccination + prednisolone) on the 43rd day (30.3 ± 8.6). The first individual with wholly epithelizated wound among the group of mice with immunosuppression was registered only on the 39th day of observation. On the 52nd day, the epithelization of the skin defect of 75% of animals was over with an average period of epithelization for these individuals 44.7 ± 4.5 days. Thus, the skin defects of mice with an active cellular immunity healed up 7.5 days earlier than controlled. This testifies to activation of regenerative-proliferative process under the influence of "Britov's vaccine". Prednisolone in the dose of 15 mg/kg/days per os causes immunosuppression, which obviously influences the wounding process and slows healing of experimental wounds of mice by more than two times in comparison with controls. Prednisolone used in conjunction with the vaccine damages the immune system less. On the basis of these experiments, one can speak about positive effects of the using "Britov's vaccine" to stimulate regenerative processes and speed the healing of skin wounds in this mouse model.

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99 Immunological response against *Trichinella spiralis* infection in rats is dose dependent

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Trichinella spiralis (*T. spiralis*) is the only known *Trichinella* out of 12 recognized species that is transmitted and maintained in both a domestic and sylvatic cycle. The *T. spiralis* sylvatic cycle involves omnivores like wild boar, carnivores like wolf and fox, and their prey animals like wild rodents. *T. spiralis* is maintained in pigs as one of the most important representatives of the domestic cycle. In Europe, Asia and Latin America, free ranging pigs of small household farms are the most important trichinellosis risk for public health. Rats, and possibly other rodents, might play a role in the transmission of *Trichinella spiralis* from domestic to sylvatic animals and *vice versa*. We studied the dynamics of *T. spiralis* infection in rats using doses ranging from very low (10 muscle larvae [ML] per rat) to very high (16,000 ML per rat). To augment the dynamics of *T.spiralis* in infected rats and to evaluate the feasibility of rats surviving high infection doses with *T.spiralis*, clinical and pathological parameters were quantified. Serological tools for detecting *T.spiralis* in rats were developed to quantitatively study the correlation between parasite load and immunological response. Results of the experimentally infected rats showed that a dose dependent antibody response was developed ranging from as low as 10 ML up to a level of 10, 000 ML. A clear positive correlation was found between the number of recovered ML and serum antibody levels, and rats that were infected with 10 or 25 ML could readily be detected by use of the *T.spiralis* western blot 2 weeks post infection, which is useful to evaluate sera from animals with low infection levels. The predictive value of measured antibody levels to estimate actual numbers of intramuscular larvae is, however, limited.

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100 TNF-α induced myocardial apoptosis and affected lipid metabolism in *Trichinella spiralis*-infected mice

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Keywords: *Trichinella spiralis*, TNF-α, apoptosis, myocardium injury, lipid metabolism, fatty acids

Mice were infected with 1000 larvae of *Trichinella spiralis* by oral infection and biochemical markers of myocardium injury were detected at different periods before and post infection. The concentration of H-FABP in serum rapidly increased from 1.72 μg/L (before the infection) to 5.01 μg/L (at 3 days post-infection). This was followed by a gradual increase reaching a peak at 9 days p.i. and a decrease to 3.73 (29 days p.i.). The sensitivity of CK-MB and cTnT was lower than the H-FABP, both of which began to increase at 9 days p.i., and reach a peak at 19 days p.i. and 24 days p.i., respectively. Morphological observation of myocardium showed that there was more serious injury of myocardium in *Trichinella spiralis*-infected mouse at from 19 days to 24 days p.i., such as rupture of muscle fibers, a lot of inflammatory cell infiltration, necrotic foci, nuclear fragmentation or disappearance, sometimes granulomatous composed of central granular cell, eosinophils, lymphocytes, macrophages and other cells, etc. The pathological changes of the myocardium in different degrees were observed during other infections.

The TNF-α, TNFR1 (TNF receptor 1), FADD (Fas-associated death domain), Caspase8, Caspase3, TRAF2 (TNF receptor associated factor-2), RIP (Receptor interactive protein), NF-κB and β-actin gene were amplified by RT-PCR. The results showed that the expression of β-actin mRNA was fairly stable and the relative expression of TNF-α mRNA was from 0.02 (before infection) to 0.88 (at 9 days p.i.), and reaching peak at 19-24 days p.i., then decreased to 0.29 at 42 p.i.; The relative expression changes of TNFR1 mRNA, FADD mRNA, Caspase8 mRNA and Caspase3 mRNA is similar to TNF-α. The concentration of TG, CHOL, HDL and LDL were detected in blood lipid of mouse before and post infection during different stages. Compared with the no infection, the average concentration of TG was higher, but the average concentration of HDL was lower. Fatty acids, including free and conjunctive fatty acids in myocardium, were extracted and methyl-estered using the

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AIB method firstly, and then the GC-MS measure was used to analyze and quantify methyl-estered fatty acids. The results indicated that the percentage of saturated fatty acids was increased obviously post infection, but the percentage of polyunsaturated fatty acids was decreased significantly. The carnitine palmitate transferase-1 (CPT-1b) was analyzed by semi-quantitative RT-PCR and the results suggested that the activity of CPT-1 was upgraded.

101 Identification of an anti-tumor protein produced by *Trichinella spiralis* using a T7 phage display library

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Trichinella spiralis (*T. spiralis*) infection confers effective resistance to tumor cell expansion. In this study, a T7 phage cDNA display library was constructed to express genes encoded by *T. spiralis*. Organic phase multi-cell screening was used to sort through candidate proteins in transfected K562 and H7402 cells using the display library. The protein encoded by the A200711 gene was identified by our screen and analyzed using protein analysis software. The molecular weight of A200711 was 16736.3, the molecular formula was identified as $C_{765}H_{1163}N_{205}O_{205}S_7$ and the hydrophobicity index was determined to be 84.04. To test the antitumor effect of A200711, cell proliferation and apoptosis variations were detected after the recombinant of pEGFP-N1-A200711 was transfected into human hepatoma cells (H7402) and normal liver cells (H7702). The results show that the expressed target gene successfully induced apoptosis in H7402 cells, as measured by Hoechst-PI staining, MTT assay and flow cytometry analysis ($p<0.05$). Apoptosis, however, was not observed in transfected H7702 cells. We speculate that the A200711 *T. spiralis* protein may contribute to the regulation of apoptosis-related genes, leading to apoptosis in H7402 tumor cells.

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102 Toll-like receptor activation by helminth or helminth products to alleviate inflammatory bowel disease

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Genetic studies have provided additional evidence to suggest that derangements in innate and adaptive immunity result in human inflammatory bowel disease (IBD). According to the hygiene hypothesis, microbes and worms are important for shaping, tuning the development and the function of the immune systems of human beings. Studies support the fact that parasitic infection must be tightly regulated to avoid severe pathology, or even mortality, by triggering TLR-dependent proinflammatory cascades. Epidemiological and experimental data have also provided further evidence that a reduction in helminth infection is linked to an increase in the incidence rate of autoimmune diseases. Helminth infection may modulate the TLR expression of dendritic cells (DCs) and responsiveness of DCs to TLR ligands, which may provoke a Th2 and/or regulatory T cell (Treg) dominant response and contribute to inflammation alleviation. Toll-like receptor (TLR) pathways are often associated with many inflammatory and autoimmune diseases. Fine control of inflammation in the TLR pathway is highly desirable for effective host defense. Thus, the use of TLR agonist from helminth or product should be considered for the treatment of inflammatory bowel disease (IBD).

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103 The Protective Effect of the Effective antigen composition of *Trichinella spiralis* on Experimental Colitis in BALB/c Mice

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Keywords: *Trichinella spiralis* soluble protein, Crohn's disease, TNBS-induced colitis, cytokines

The *hygiene hypothesis* suggests an inverse relationship between the incidence of parasitic infections and chronic inflammatory bowel diseases (IBD). The excretory-secretory proteins of helminthes play an important role in the process of immunomodulation. Aims In the present study, we investigated the therapeutic potential of *T.s (Trichinella spiralis)* soluble proteins (WN10,ZH68,T668, WM5) on experimental colitis in mice. BALB/c mice were treated subcutaneously with 50mg soluble proteins three times at an interval of 10 days. Colitis was induced by intra-rectal administration of 5 mg trinitrobenzene sulfonic acid (TNBS) in 50% ethanol. Disease activities and macroscopic and microscopic scores were evaluated. To determine immune response provoked by soluble proteins, inflammation score, extent of inflammation, and myeloperoxidase (MPO) activity. To determine immunological pathways induced by *Trichinella* spp. proteins we measured cytokine profiles of T-lymphocytes from colon, mesenteric lymphnodes (MLN), and spleen by real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Control mice showed no signs of inflammation, whereas all inflammatory parameters were significantly increased in mice with colitis. Treatment of mice with colitis with *T.s* proteins ameliorated significantly the disease activity index (DAI) as well as the macroscopic and microscopic scores and MPO activity. Immunologically, induction of colitis significantly increased expression of IFN- mRNA in the inflammed colon. Treatment with *S. mansoni* proteins caused a decrease of proinflammatory cytokines (IFN-γ, IL-17, IL-23) in colon and MLN, whereas the production of regulatory cytokines (IL-10, TGF-β) increased significantly in colon tissue. Treatment with proteins of *Trichinella* spp. proteins ameliorated TNBS-induced colitis in mice. We interpret these findings to indicate that infection of mice with *T.s* distracts the

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mucosal immune system response from a Th1 response toward a protective Th2 response with an attendant reduction in the severity of colonic damage and balance of Th1/Th2. Therefore, we suggest a therapeutic potential for helminth proteins in the treatment of IBD.

104 Differential immunological responses induced by infection with female adult worms or new born larvae of *Trichinella pseudospiralis*

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Keywords: *Trichinella pseudospiralis*, developmental stage, immunological response

Trichinella pseudospiralis infection can modulate the immunological response of autoimmune and allergic diseases, leading to the amelioration of these diseases experimentally. The present study was undertaken to determine what stage of the parasite may be responsible for the immunological modulation, by comparing the peripheral eosinophilia, IgE and IgG antibody response, and cytokine profile between the infection of female adult worm and newborn larva. Higher eosinophilia was observed in new born larva infection which than in female adult worm infections, while much higher IgE level was observed in adult worm infection than in muscle larva infection. The responses of IgG1, IgG2a and IgG2b to different antigens, including adult crude, muscle larva crude and ES antigens, were investigated. IgG1 response to ES antigen was predominant in adult worm infection. IgG2a and IgG2b responses to larva crude antigen were predominant in newborn larva infection. Productions of IFN-gamma and IL-4 were observed after restimulation of splenocytes from infected mice with adult crude, muscle crude and ES products. The IL-4 cytokine was predominant in response to the restimulation of adult crude and ES product in adult worm infection. No significant differences were observed in the levels of IFN-gamma between adult worm and new born larva infections. The present study demonstrated that adult worm and muscle larva induced different immunological responses, and adult worm induced higher Th2 response than muscle larva did. Further determination of the modulation mechanism may be helpful for identifying the molecules from *Trichinella* responsible to the immunological regulation in the amelioration of autoimmune and allergic diseases.

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105 The effect on SP2/0 myeloma cell by *Trichinella spiralis* TS2 recombinant protein *in vivo*

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Keywords: TS2, SP2/0, *T. spiralis*, associated antigen

Trichinella spiralis TS2 recombinant protein is an associated antigen protein with SP2/0 myeloma cell. To observe anti-tumor effect of TS2 recombinant protein on SP2/0 myeloma *in vivo*, the prokaryotic expression plasmid pET-28a-TS2 has been constructed. BALB/c mice were divided 4 groups randomly. The mice in two groups were immunized with the TS2 recombinant protein every twice daily for 10 days by either intraperitoneal or intramuscular injection. At the same time, the other two groups were immunized with immunoadjuvant or phosphate-buffered saline as control. All groups of the mice were inoculated by SP2/0 myeloma cells (2×10^6) at third day after the final immunization, the anti-tumor effect was evaluated by humoral, cellular immune and the rate of inhabit tumor 30 days later. The results showed that the group immunized with TS2 recombinant protein developed significant increases of the percentages of $CD4^+$, $CD8^+$ and $CD19^+$ cells compared with the control groups ($p<0.05$), but there is no significant difference between intraperitoneal and intramuscular injection ($p>0.05$). Also we found that there was no difference between the weight or size of tumor in intraperitoneal and intramuscular groups ($p>0.05$), but they are obviously lighter than control groups ($p<0.05$). The results indicated that *Trichinella spiralis* TS2 protein may have a role in inhibit tumor growth.

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106 Growth Suppression Effects and Identification of The Differentially Expressed Genes by Infected with *Trichinella spiralis* Induced on SP2/0 Myeloma

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Keywords: *Trichinella spiralis*, SP2/0 Myeloma Cell, suppression subtractive hybridization

To research the molecular mechanism of SP2/0 myeloma cell growth suppression in mice infected with *Trichinella spiralis*, a model was established in Balb/c mice infected with *Trichinella spiralis* and the induced tumor cells growth were inhibited. We used the technique of suppression subtractive hybridization (SSH) for identifying the differentially expressed genes between uninfected and infected tumor cells. On the 11th day, 2×10^6 myeloma cells were inoculated subcutaneously into 6 to 8-week-old BALB/c female mice, some of which had been innoculated with 450 larvae of *Trichinella spiralis*. They were killed on the 28th day after inoculation with cancer cells. Tumors were measured with a dial caliper, and the tumor volume was calculated. Total RNA and mRNA of tumor tissue was prepared. A subtracted cDNA library of infected and induced with *Trichinella spiralis* was constructed by SSH. The differentially expressed genes were identified using the reverse Northern Blot technique. The positive clones were sequenced and the homology searched in the GenBank nr and EST databases by BLASTn. The results showed that the part of related genes expression cDNA subtracted library had been successfully constructed by *Trichinella spiralis* induced SP2/0 myeloma Cell. The 20 randomly selected positive clones were identified by PCR. The results indicated that the inserted fragments ranged between 180-850bp.Therefore, the function of MRPL41, NKTR, RbAp48, QRS and ANXA2 might relate to tumor inhibiting effects. They might play an important role in the process by which *Trichinella spiralis* suppresses tumors in this mouse model.

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107 Anti-tumor Effect of Antibody against Associated Antigens between Hela Cell and *Trichinella spiralis*

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Keywords: *Trichinella spiralis*, Hela cell, antibody

The ability of an antigen derived from *Trichinella spiralis* to induce tumor suppression in mice subsequently administered Hela cells was investigated. The antibody of associated antigens between Hela cell and *Trichinella spiralis* were determined by ELISA and Western blotting. The 40 KDa associated antigen between Hela cell and *Trichinella spiralis* were collected by GST column. BALB/C mice were immunized by the associated antigen by conventional means. Serum was collected while the titer was up to 1:6400. Nude mice were inoculated with Hela cells (4×10^6). Twenty days later after inoculated, the nude mice were divided into A, B, C, D, E, F, G groups. The mice in group A,B,C were intraperitoneally injected with 0.2mL containing 25μg, 35μg, 45μg antibody of anti-*Trichinella spiralis* soluble antigen every two days for successive 5 times respectively. In same way, the mice in groups D, E, F were treated with 0.2mL 25μg, 35μg, 45μg antibody of the associated antigens. The mice in group G were treated with PBS as control. All mice were necropsied and the rates of tumor inhibition were calculated. The result showed that the rate of inhibition tumor treated with antibody of anti-*Trichinella spiralis* soluble antigen and associated antigens was 52.2% and 82.3% respectively. So the antibody of associated antigens may have anti-tumor effect.

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108 *Trichinella spiralis* infection in the large intestine follows a distinct profile compared to infection of the small intestine

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Keywords: *Trichinella spiralis*, large intestine, mast cells, IL-10

Trichinella spiralis is well known as a parasite of the small intestine of vertebrates. However, this nematode also invades a secondary location, the large intestine, which has not been well studied. It is known from experiments using multiple models of intestinal inflammation that the small intestine and the large intestine constitute distinct immune environments. The aim of this study was to evaluate differences between the small intestine and large intestine during infection of rodents with *T. spiralis*. Our results show that in C57Bl/6 mice, parasites migrate distally, arriving first in the small intestine, with peak infection of the cecum at day 9 and a peak infection of the large intestine at day 13. The distribution of male and female parasites differed among these sites. Although a strong mastocytosis is induced in the small intestine of infected mice, mastocytosis did not occur during infection of the large intestine. The immunosuppressive cytokine IL-10 contributes to the control of intestinal inflammation, and we have evaluated differences in the role of IL-10 between the small and large intestine. Although *Trichinella* is closely related to the cecal dwelling nematode *Trichuris*, immune responses that expel the two organisms are distinctive. Differences may result from variation in parameters of the two life cycles or from the location of the habitats occupied by the two worms. Comparing immunity in the small and large intestine, within a single infection, will allow us to isolate factors that are crucial to immunity at each site. Insights into the differences in the induction and control of inflammation between the small and large intestine may also help us understand the etiology of inflammatory bowel diseases.

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109 *Trichinella spiralis* infection during human pregnancy. A case report

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***Keywords**:* human trichinellosis, pregnancy, helminthocytotoxic activity, newborn larvae, specific immunoglobulins isotypes, IgE

Infection by *Trichinella* spp. during pregnancy still raises many questions. In Argentina, where trichinellosis is endemic, an important number of outbreaks have occurred over time where pregnant women might be involved. The aim of this work was to study the clinical, laboratory and inmunoserological (IS) features of trichinellosis in an infected pregnant woman and her newborn. The patient was a 24-year-old infected woman in her second trimester of pregnancy (P).She acquired the infection through the ingestion of pork salami from a commercial source. After the first week post-infection (pi) she presented a strong periorbitary oedema without conjunctival injection, mild myalgia, nauseas and vomits. Hospitalization was not necessary. At the time of serological testing, day 30 pi, slightly elevated muscle enzymes were observed (CPK: 180 UI/l, LDH: 397 UI/l, total leukocytes count 8300/mm^3 and eosinophilia 19%. The IS diagnosis by indirect immunofluorescence (IIF) and ELISA detected specific antibodies (Abs). The patient gave birth to a healthy newborn through full-term. Serum samples from the infected mother at day 30 pi (MoP) and at delivery; 169 days pi (MoD), newborn and cord blood samples were collected. Detection of specific Abs against excretory-secretory products of muscle larvae of *T. spiralis* (ML-ESP) was carried out in all serum samples by ELISA. Anti ESP-LM-total immunogammaglobulins (Ig), IgG and IgE were detected in all sera analyzed, IgM and IgG4 in Mo samples and IgA only in MoP serum. Total Ig and IgE antibodies against newborn larvae surface (NBLS) were detected by IIF in all serum samples. All sera samples analyzed showed helminthotoxic activity against NBLS by *in vitro* Dependent Cell Cytotoxicity (ADCC) assay (MoP 33%, MoD 27%, Cord 32%, Infant 24%, cytotoxic serum 60% and normal serum 0%). The parasite burden of swine product samples (artificial digestion) was 1 ML/g. The isolated ML was *Trichinella spiralis*, as determined by multiplex PCR assays at the International *Trichinella* Reference Centre. The mild symptoms and signs presented by the patient might be associated to the low parasite burden of the ingested product and pregnancy status. The presence of helminthocytotoxic Abs against NBLS in the patient's serum, together with the increase in eosinophils in blood, suggest a decrease in muscle parasite burden and might reduce the possibility of infecting the fetus in case of transplacental passage of the larvae. The detection of IgE in the cord serum and in the newborn suggests the existence of IgE trans-placental passage.

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110 Digital gene expression analysis of three life cycle stages of the zoonotic parasite *Trichinella spiralis*

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Keywords: Digital gene expression, *Trichinella spiralis*, gene ontology, transcriptome

Digital gene expression (DGE) analysis offers a route to detect gene expression differences. To better understand the genes involved in the development of *Trichinella spiralis* (*T. spiralis*), we compared the gene expression variations among three life cycle stages using next generation sequencing technology. In this study, almost 15 million tags were generated, producing expression data for more than 4,000 genes, covering 50% of the genes in the genome. It was found that more than 20% of the genes were transcribed from both strands. Thousands of genes showed significantly diverse expression levels based on the various comparisons. Further, based on gene ontological analysis, over 600 genes were functionally categorized and biological pathways that are differentially functional in three life cycle stages were elucidated. Then we randomly selected some genes to confirm the differential expression in three stages of the life cycle by quantitative real-time PCR (qRT-PCR). Overall, many metabolic and biological pathways have been identified and the data showed predicted patterns of gene expression profiles and molecular evolution in the T. spiralis genome. Our study highlighted the usefulness of DGE profiling data for quantifying gene expression at the transcriptional level and could facilitate our understanding of the molecular mechanisms from various physiological aspects.

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