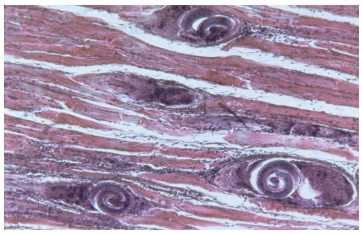
Abstracts

14th International Conference on Trichinellosis

Berlin, Germany
14–18 September 2015

International Commission
on Trichinellosis



organized by the Federal Institute for Risk Assessment (BfR) in cooperation with
The Free University Berlin (FUB),
The Section of Parasitology of the German Society of Veterinary Medicine (DVG), and
The Federal Ministry of Food and Agriculture (BMEL)

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14th International Conference on Trichinellosis

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Welcome Address

Professor Dr. Dr. Andreas Hensel

President
Federal Institute for Risk Assessment (BfR)

Trichinellosis is an important foodborne zoonosis which remains a public health concern in several regions of the world and therefore requires appropriate control actions. First activities for the systematic control of trichinellosis date back to the 1860s in Germany, where Rudolf Virchow (1821–1902), the pioneer of modern food hygiene, initiated the mandatory *Trichinella* testing in pigs at slaughter houses.

We are proud to organize and host the 14th International Conference on Trichinellosis in cooperation with the Faculty of Veterinary Medicine of The Free University Berlin (FUB), the Section of Parasitology of the German Society of Veterinary Medicine (DVG) and the Federal Ministry of Food and Agriculture (BMEL) and to continue the good tradition from the preceding international conferences which were held in Changchun (China), Plitvice Lakes (Croatia) or San Diego (USA).

I am delighted to welcome you at this conference here in Berlin, the capital city of Germany, which was the home to so many Nobel laureates. For instance, here Robert Koch was the first to identify pathogenic microorganisms as the cause of zoonotic diseases such as tuberculosis, anthrax and cholera. Robert Koch was probably the most famous staff member of the Imperial Health Agency which is the predecessor of our institution, the Federal Institute for Risk Assessment.

Berlin was documented for the first time in the 13th century and is a city of culture, politics, media and science. Just last year, the citizens of Berlin celebrated the 25th anniversary of the fall of the Berlin Wall, symbolising the reunification of Germany after the Second World War.

From 14th to 18th September 2015 my team and I will do everything to ensure your pleasant stay in Berlin and a successful conference at the Seminaris Campus Hotel. It is a great honor for us to welcome the *Trichinella* research community and I wish you an inspiring meeting with fruitful discussions.

Welcome Address

Dr. Maria Flachsbarth

Parliamentary State Secretary
Federal Ministry of Food and Agriculture (BMEL)

Zoonoses are diseases which are naturally transmissible directly or indirectly between humans and animals. Trichinellosis is a major foodborne zoonosis that occurs throughout the world.

The fact that humans are still affected by serious, sometimes lethal, trichinellosis suggests the latent risk due to *Trichinella* species that exists on a number of continents. This underlines the importance of scientific research and in particular of cross–border technical exchange and cooperation on biology, epidemiology, diagnostics, therapy and on preventive measures to effectively tackle this risk.

This cross–border exchange of experience regarding prevention, control and current knowledge was one of the objectives when founding the International Commission on Trichinellosis in 1958. The Commission successfully took the first steps towards realising this objective at the 1st International Conference on Trichinellosis in Warsaw in 1960, and has since continued its progress at other international conferences, most recently in Croatia in 2007 and in China in 2011.

It is a pleasure for me to welcome you today to the 14th International Conference on Trichinellosis, which has been organised by the Federal Institute for Risk Assessment on behalf of the International Commission on Trichinellosis, and supported by the German-Veterinary Asociation, the Free University of Berlin and the Federal Ministry of Food and Agriculture. I am convinced that the challenging programme which includes key contributions from Argentina, Australia, Denmark, France, the United Kingdom and the United States, will continue to develop this successful scientific exchange.

I wish you every success at this conference and hope that the stimulating discussions result in new ideas for successfully controlling, preventing and fighting this zoonotic disease!

Content

1 Program of the International Conference on Trichinellosis

1.1 Program for oral presentations

Tuesday, 15th September 2015

Session I	**Phylogeny, Genomics and Proteomics** Chairs: Dante Zarlenga (USA), Guadalupe Ortega–Pierres (Mexico)
09:45–10:10 hrs	**Keynote: Benjamin Rosenthal** Phylogeny and comparative population biology of *Trichinella* spp.
10:10–10:45 hrs	**Keynote: Robin Gasser** Genomes of all members of the *Trichinella* complex: phylogenomic and biogeographic reconstruction
10:45–11:00 hrs	**Frits Franssen, Ewa Bilska–Zając, Gunita Deksne, Hein Sprong, Edoardo Pozio, Benjamin Rosenthal, Mirek Rozycki, Joke van der Giessen** Interspecies recombination between *Trichinella spiralis* and *Trichinella britovi* occurs under natural conditions
Session II	**Phylogeny, Genomics and Proteomics** Chairs: Dante Zarlenga (USA), Guadalupe Ortega–Pierres (Mexico)
11:30–11:45 hrs	**G. Ortega–Pierres, L.E. Grijalva–Contreras, R. Fonseca–Liñán, Guillermo Mendoza, V. Flores–López, R.M. Bermúdez–Cruz** Proteomic analysis of differentially expressed proteins from muscle larvae and pre–adult stages of *Trichinella spiralis*
11:45–12:00 hrs	**Dante Zarlenga, Zhengyuan Wang, Makedonka Mitreva** *Trichinella spiralis*; adaptation and parasitism
12:00–12:15 hrs	**Martin Kašný, Lucie Škorpíková, Jana Ilgová, Břetislav Koudela, Milan Gelnar, David Potěšil, Zbyněk Zdráhal, Peter Thompson, Dante Zarlenga** The comparison of functional molecules from excretory–secretory products of two *Trichinella* species
12:15–12:30 hrs	**X.L. Liu, Y. Wang, M.Y. Liu, X. Bai, H. Gao, X.P. Wu, T.T. Li, H.N. Shi, P. Boireau, B. Rosenthal, X.L. Wang** Immunoproteomic analysis of the excretory–secretory products of the *Trichinella pseudospiralis* adult and newborn larvae
12:30–12:45 hrs	**Peter C. Thompson, Luke Hecht, Dante S. Zarlenga, Benjamin M. Rosenthal** Assortative mating limits hybridization between two lineages of freeze–resistant *Trichinella*
Session III	**Biology, Host–Pathogen–Interaction and Immunology** Chairs: Isabelle Vallee (France), Liu Mingyuan (China)
14:30–14:55 hrs	**Keynote: Bernadette Connolly** The biology of *Trichinella*: from genes to genomes
14:55–15:10 hrs	**Natasa Ilic, Alisa Gruden–Movsesijan, Jelena Cvetkovic, Marija Devic, Sasa Vasilev, Ljiljana Sofronic–Milosavljevic** Individual components of *Trichinella spiralis* excretory–secretory muscle larvae antigens – the role in shaping of the immune response

Tuesday, 15th September 2015

Session III	Biology, Host–Pathogen–Interaction and Immunology Chairs: Isabelle Vallee (France), Liu Mingyuan (China)
15:10–15:25 hrs	**Alisa Gruden–Movsesijan, Natasa Ilic, Zanka Bojic–Trbojevic, Danica Cujic, Milica Zekovic, Ljiljana Sofronic–Milosavljevic** The presence of galectin–1–like molecules in *Trichinella spiralis* muscle larvae excretory–secretory antigens
15:25–15:40 hrs	**Julia Dąbrowska, Monika Dybicz, Maria Doligalska** The ultrastructural analysis of pulmonary oedema formation in BALB/c mice infected with *Trichinella spiralis*
15:40–15:55 hrs	**F. Bruschi, C. Della Bella, M. Benagiano, M. De Gennaro, M.A. Gomez–Morales, A. Ludovisi, S. Luchi, E. Pozio, M.M. D'Elios** T cell clones in human trichinellosis: Th2 polarization

Wednesday, 16th September 2015

Session I	Biology, Host–Pathogen–Interaction and Immunology Chairs: Isabelle Vallee (France), Liu Mingyuan (China)
09:00–09:25 hrs	**Keynote: Pascal Boireau** Host–pathogen interaction – the features of *Trichinella* antigens
09:25–09:40 hrs	**Maria Doligalska, Klaudia Brodaczewska, Natalia Wolaniuk** The inflammation induced by *N*–acetyl–glucosamine and glucosamine supports *T. spiralis* infection
09:40–09:55 hrs	**Bao–QuanFU, Zi–Gang QU, Xue–Ting MA, Wen–Hui LI, Nian–Zhang ZHANG, Long YUE, Jian–Min CUI, Wan–zhong JIA, Jian–Ping CAI** Molecular cloning and characterization of a cathepsin–F–like protease from *Trichinella spiralis*
09:55–10:10 hrs	**J. Ding, M.Y. Liu, X. Bai, S.M. Sun, X.P. Wu, Y. Wang, J. Liu, P. Boireau, B. Rosenthal, X. L. Wang, X.L. Liu** Anti–tumor effects produced by excretory–secretory products of *Trichinella spiralis*
10:10–10:25 hrs	**M.Y. Liu, X.L. Liu, C.S. Liao, X. Bai, X.P. Wu, P. Liu, H.N. Shi, P. Boireau, B. Rosenthal, X.L. Wang** *Trichinella spiralis* p43, a Deoxyribonuclease Ⅱ but no activity
10:25–10:40 hrs	**X. Bai, M.Y. Liu, X.P. Wu, H.N. Shi, P. Boireau, B. Rosenthal, X.L. Wang, X.L. Liu** The roles of macrophage treated by excretory–secretory products from muscle larvae of *Trichinella spiralis* on the differentiation of C2C12 myoblasts
10:40–10:55 hrs	**Jing Cui, Peng Jiang, Zi Fang Zhang, Xi Zhang, Ruo Dan Liu, Ge Ge Sun, Xin Qi, Li Na Liu, Zhong Quan Wang** New insights into the mechanism of *Trichinella spiralis* infective larvae invade the intestinal epithelium

Wednesday, 16th September 2015

Session II	**Detection** Chairs: Ljiljana Sofronic–Milosavljevic (Serbia), Antti Oksanen (Finland)
11:30–11:55 hrs	**Keynote: Mabel Ribicich** *Trichinella spiralis* and *Trichinella patagoniensis* – how to improve their detection in endemic regions?
11:55–12:10 hrs	**Judith A. Appleton, Lucille F. Gagliardo, Dolores E. Hill, Maria A. Gomez–Morales, Elizabeth A. Berliner, Brian P. Butler** Detection of antibodies specific for *Trichinella spiralis* by competition: one assay for a variety of animal hosts
12:10–12:25 hrs	**Andreas Latz, Jenny Völger, Karin Fehr, Lidia Chitimia, Helmut Duchmann** Development and performance evaluation of enzyme linked immunosorbent assay and lineblot for serological diagnosis of *Trichinella* in humans and animals
12:25–12:40 hrs	**Zhong Quan Wang, Ge Ge Sun, Ruo Dan Liu, Peng Jiang, Xi Zhang, Li Wang, Jing Cui** Early serodiagnosis of trichinellosis by ELISA using excretory–secretory antigens of *Trichinella spiralis* intestinal infective larvae
Session III	**Detection** Chairs: Ljiljana Sofronic–Milosavljevic (Serbia), Antti Oksanen (Finland)
14:00–14:15 hrs	**Alvin Gajadhar, Kelly Konecsni, Brad Scandrett, Cheryl Scheller** Evaluation of sample storage conditions for the recovery of *Trichinella* larvae from pork and horsemeat by the artificial digestion method
14:15–14:30 hrs	**Nikol Reslová, Martin Kašný, Michal Slaný, Břetislav Koudela, Petr Králík** Diagnostic panel for foodborne pathogens
14:30–14:45 hrs	**Antti Oksanen, Kristian Björnstad, Anu Näreaho, Anna Lundén, Ulf Bondesson** Faster and cheaper species identification of *Trichinella* muscle larvae
14:45–15:00 hrs	**A. Mayer–Scholl, J. Murugaiyan, J. Neumann, P. Bahn, S. Reckinger, K. Nöckler** Rapid detection of the foodborne pathogen *Trichinella* spp. by MALDI–TOF mass spectrometry
15:00–15:15 hrs	**B. Tang, X.L. Liu, M.Y. Liu, X.P. Wu, Y. Wang, H.N. Shi, P. Boireau, B. Rosenthal, X. Bai, X.L. Wang** Epitope mapping of a strongly antigenic cystatin–like protein from *Trichinella spiralis*
15:15–15:30 hrs	**Jenny Chaparro-Gutiérrez, Anderson López-Arias, Sara López-Osorio, Corina Zambrano-Moreno, Jaime Mejia-Jaramillo, Felipe Penagos-Tabares, Diego Piedrahita** Initial examination of 6 pig slaughterhouses and a sample of wild rodents have not detected *Trichinella* in different towns of Antioquia, Colombia

Thursday, 17th September 2015

Session I	**Epidemiology** Chairs: Edoardo Pozio (Italy), Joke van der Giessen (Netherlands)
09:00–09:25 hrs	**Keynote: Darwin K. Murrell** The dynamics of *Trichinella spiralis* epidemiology: From down on the farm to living wild?
09:25–09:40 hrs	**Edoardo Pozio** The epidemiology of *Trichinella pseudospiralis* an elusive nematode
09:40–09:55 hrs	**Alessandra Ludovisi, Marco Selmi, Maria Angeles Gómez Morales, Marco Amati, Eleonora Fiorentino, Lorenzo Breviglieri, Giovanni Poglaye, Edoardo Pozio** Hunting dogs as sentinel animals for monitoring *Trichinella* spp. infection in wildlife
09:55–10:10 hrs	**Karsten Nöckler, Sabine Reckinger, Anne Mayer–Scholl, Christoph Schulze** Study on the prevalence of *Trichinella* spp. in raccoon dogs (*Nyctereutes procyonoides*) in Brandenburg, Germany
10:10–10:25 hrs	**Gianluca Marucci, Giuseppe La Rosa, Isabelle Vallee, François Casabianca, Pascal Boireau, Edoardo Pozio** Microsatellite analysis of *Trichinella britovi* isolates from the Mediterranean islands of Corsica and Sardinia suggests their different geographical origin
Session II	**Epidemiology** Chairs: Edoardo Pozio (Italy), Joke van der Giessen (Netherlands)
11:00–11:15 hrs	**Kristina Roesel, Michel Dione, Karsten Nöckler, Reinhard Fries, Maximilian P.O. Baumann, Peter–Henning Clausen, Delia Grace** Exposure of pigs to *Trichinella* spp. in three districts in Central and Eastern Uganda
11:15–11:30 hrs	**I. Jahundoviča, G. La Rosa, M. Kirjušina, D. Tonanzi, E. Pozio** Microsatellite analysis of *Trichinella britovi* isolates from Latvia
11:30–11:45 hrs	**G. La Rosa, R. Calero–Bernal, J.E. Pérez–Martín, J.A. Gamito, D. Tonanzi, F.J. Serrano–Aguilera, E. Pozio** Microsatellite analysis of *Trichinella spiralis* gene pool circulating in the Extremadura region of Spain
11:45–12:00 hrs	**Călin Gherman, Dan Neagu, Miruna Oltean** Wild carnivores, a major component of sylvatic reservoir for *Trichinella* spp. in Romania
12:00–12:15 hrs	**Isabelle Vallee, Michele Riera, Céline Richomme, Sandrine Lacour, Gina Zanella, François Casabianca, Pascal Boireau** *Trichinella* in Corsica Island: when the parasite takes advantage of the slightest weak link
12:15–12:30 hrs	**Alex Markovics, Asael Roth, Roni King, Oran Erster** Prevalence and molecular characterization of *Trichinella* infection in wild animals in Israel

Thursday, 17th September 2015

Session III	**Human Trichinellosis and Treatment** Chairs: Fabrizio Bruschi (Italy), Bozena Moskwa (Poland)
14:00–14:25 hrs	**Keynote: Jean Dupouy–Camet** Trichinophobia in Europe in the XIXth century
14:25–14:40 hrs	**Maria Angeles Gomez Morales, Marco Amati, Alessandra Ludovisi, Giovanni Mazzarello, Claudio Viscoli, Edoardo Pozio** Inference of the *Trichinella* species causing a human outbreak by serology
14:40–14:55 hrs	**Codruta Nemet, Cristina Dobrescu, Mihaela Idomir, Lavinia Buvnariu, Eugen Ionescu** The importance of objective clinical examination in patients with trichinellosis, registered in Braşov County, Romania, between 1983–2013
14:55–15:10 hrs	**Mihaela Idomir, Codruta Nemet, Cristina Dobrescu, Mihaela Emandi, Lavinia Buvnariu** The socio–professional configuration of trichinellosis patients for a period of 30 years
15:10–15:25 hrs	**Natasa Miladinovic–Tasic, Ljiljana Sofronic–Milosavljevic, Sasa Vasilev, Nenad Ristovic, Marija Devic, Suzana Otasevic** Serological diagnosis of trichinellosis during two outbreaks in the territory of southeast Serbia
Session IV	**Human Trichinellosis and Treatment** Chairs: Fabrizio Bruschi (Italy), Bozena Moskwa (Poland)
16:00–16:15 hrs	**Sasa Vasilev, Sanja Celebicanin, Tamara Boskovic, Bojana Grgic, Jelena Cvetkovic, Milovan Djordjevic, Ljiljana Sofronic–Milosavljevic** *Trichinella* infection in Serbia from 2011 to 2014
16:15–16:30 hrs	**Leen Claes, Marjan Van Esbroeck, Peter Messiaen, Annemarie Forier, Pierre Dorny** An outbreak of trichinellosis in Belgium associated with the consumption of imported wild boar meat
16:30–16:45 hrs	**Polya Rosin, Orla Condell, Mathieu Bangert, Johanna Takkinen and the Expert Panel for food– and waterborne parasitic diseases** Perceived barriers to ascertainment and reporting of human trichinellosis cases in the European Union/European Economic Area
16:45–17:00 hrs	**A. Malakauskas, A. Bartulienė, V. Paulauskas, M. Malakauskas** Epidemiology of trichinellosis in Lithuania 2002–2014
17:00–17:15 hrs	**R. Blaga, C. Gherman, B. Durand, P. Boireau, C. Cretu** Romania and trichinellosis: a never ending story

Friday, 18th September 2015

Session I	**Legislation and Control** Chairs: Ray Gamble (USA), Leen Claes (Belgium)
09:00–09:25 hrs	**Keynote: Lis Alban** How to ensure a negligible risk of *Trichinella* in pig farming from a control perspective?
09:25–09:40 hrs	**P. Buholzer, X. Yang, M. Pürro, T. Tarjan, K. Hansen, A.J. Raeber** Development of an alternative artificial digestion method for detecting *Trichinella* larvae in meat of domestic swine
09:40–09:55 hrs	**M. Ribicich, M. Pasqualetti, F. Fariña, N. Cardillo, A. Rosa, M. Acerbo, M. Miguez, O.J. Degregorio** Trichinellosis and pork production in Argentina: New aspects of control in an endemic region
09:55–10:10 hrs	**Kelly Konecsni, Cheryl Scheller, Brad Scandrett, Alvin Gajadhar** Evaluation of the PrioCHECK digestion assay kit for the detection of *Trichinella* larvae in pork, horsemeat and wildlife
10:10–10:25 hrs	**Frits Franssen, Arno Swart, Katsuhisa Takumi, Arie Havelaar, Joke van der Giessen** From parasite to patient: a quantitative risk analysis model for *Trichinella*
Session II	**Legislation and Control** Chairs: Ray Gamble (USA), Leen Claes (Belgium)
11:00–11:15 hrs	**Patrizia Rossi, Ray Gamble, Pascal Boireau, Brent Dixon, Alvin Gajadhar, Elisa Goffredo, Karsten Nöckler, Edoardo Pozio, Isabelle Vallee, Joke van der Giessen, Paul Vanderlinde** EN ISO 18743: Detection of *Trichinella* larvae in meat by artificial digestion method: the first international standard on parasites in food microbiology
11:15–11:30 hrs	**Brigitte Hentrich, Caroline F. Frey, Gertrud Rosenberg, Bruno Gottstein** Introduction of the Latex–agglutination test in Switzerland for the detection of *Trichinella* infections in pigs
11:30–11:45 hrs	**Caroline F. Frey, Brigitte Hentrich, Marie–Pierre Ryser Degiorgis, Manon Schuppers, Bruno Gottstein** Epidemiological situation of *Trichinella* and control measures in Switzerland
11:45–12:00 hrs	**Joke van der Giessen, Lucy Robertson** Ranking of *Trichinella* species and other foodborne parasites
12:00–12:15 hrs	**Miroslaw Różycki, Ewa Bilska–Zając, Ewa Chmurzyńska, Jacek Karamon, Tomasz Cencek** Retrospective analysis of proficiency testing results of Polish laboratories performing *Trichinella* examination of pig meat
12:15–12:30 hrs	**G. Zanella, L.A. Ndounga Diakou, A. Heckmann, P. Macé, P. Boireau, I. Vallee** Is mainland France free from *Trichinella* infection of domestic pigs?

Friday, 18th September 2015

Session III	**Student Research Award** Chairs: Alvin Gajadhar (Canada), Karsten Nöckler (Germany)
14:00–14:30 hrs	**M.P. Saracino, M.A. Calcagno, E. Bilen Beauche, A. Garnier, C.C. Vila, R. Taus, S.M. Venturiello** *Trichinella spiralis* infection and transplacental passage in human pregnancy. Differences between low versus high parasite burden areas?
14:30–15:00 hrs	**G. Makrutzki, M. Koethe, A. Hamedy, E. Lücker** Automated digital imaging and analysis system for detection of *Trichinella* larvae in meat

1.2 Program for poster presentations

Tuesday, 15th September 2015

13:30–14:30 hrs	**Phylogeny, Genomics and Proteomics**
PGP–1	**Ewa Bilska–Zając, Mirosław Różycki, Frits Franssen, Joke van der Giessen, Ewa Chmurzyńska, Tomasz Cencek** Molecular characterisation of Trichinella larvae isolated from wild boars with correlation to geographical origin of host
PGP–2	**Bao–QuanFU, Jin–Yi LIU, Nian–Zhang, Wen–Hui LI, Hong–Bin YAN, Yang YANG, Zi–Gang QU, Jian–Min CUI** Comparative Proteomics analysis of three developmental stages of *Trichinella spiralis*
PGP–3	**Justyna Bień, Anu Näreaho, Pekka Varmanen, Katarzyna Goździk, Bożena Moskwa, Władysław Cabaj, Tuula A Nyman, Kirsi Savijoki** Fluorescent two–dimensional difference gel electropho–resis and mass spectrometry for the identification of species–specific *Trichinella spiralis* and *T. britovi* antigens
PGP–4	**X.L. Liu, Y. Wang, M.Y. Liu, X. Bai, Y. Sun, X.P. Wu, H.N. Shi, P. Boireau, B. Rosenthal, X.L. Wang** Identification of *Trichinella spiralis* early antigens from excretory–secretory products of adult and newborn larvae by two–dimensional gel electrophoresis and immune–blotting
PGP–5	**Irina M. Odoyevskaya, Ivan V. Seriodkin, Alexander V. Uspensky, Irina J. Filippova, Sergei O. Movsessian, Jylia Rudenskaya** Adaptive properties and activity of proteolitic enzymes of the Arctic isolates of *Trichinella* under experimental inoculation of laboratory rodents
PGP–6	**Irina M. Odoyevskaya, Ivan Pavlasek, Sergei E. Spiridonov** Modified primers 37F and 42R for amplification of cytochromoxydase I mitochondrial gene of *Trichinella*
PGP–7	**Zhong Quan Wang, Ruo Dan Liu, Jing Cui, Ge Ge Sun, Xi Zhang, Peng Jiang, Li Wang,** Screening and identification of early diagnostic antigens from *Trichinella spiralis* intestinal infective larvae by immunoProteomics
PGP–8	**Jing Cui, Ruo Dan Liu, Ge Ge Sun, Peng Jiang, Xi Zhang, Li Wang, Zhong Quan Wang** Immunoproteomic profile of *Trichinella spiralis* adult worm excretory–secretory antigens recognized by early infection sera

Tuesday, 15th September 2015

13:30–14:30 hrs	**Biology**
BIO–1	**Bao–QuanFU, Zi–Gang QU, Long YUE, Xue–Ting MA, Wen–Hui LI, Nian–Zhang, Jian–Min CUI, Wan–zhong JIA, Jian–Ping CAI** Cloning and bioinformatics analysis of Thioredoxin peroxidase gene TsTPx1–3 from *Trichinella spiralis*
BIO–2	**Bao–QuanFU, Nian–Zhang, Jin–Yi LIU, Wen–Hui LI, Yang YANG, Jian–Min CUI Hong–Bin YAN, Zi–Gang QU** Cloning and identification of a putative aquaporin from *Trichinella spiralis* (TsAQP)
BIO–3	**Dolores E. Hill, Dante S. Zarlenga, Joseph F. Urban Jr.** Inactivation of encysted muscle larvae in pigs using Mebendazole
BIO–4	**M. Pasqualetti, F. Fariña, A. Rosa, N. Cardillo, M. Ribicich** Infectivity of *Trichinella spiralis* muscle larvae recovered from pig carcasses
BIO–5	**Zhong Quan Wang, Jing Cui , Wei Yang, Shuai Bing Zhang, Ruo Dan Liu, Xi Zhang, Peng Jiang, Shao Rong Long, Hui Jun Ren** DsRNA–mediated silencing of Nudix hydrolase in *Trichinella spiralis* inhibits the larval invasion and survival in mice

Wednesday, 16th September 2015

13:00–14:00 hrs	**Host–Pathogen–Interaction and Immunology**
HPI–1	**X. Bai, M. Y. Liu, X.P. Wu, Y.F. Wang, H.N. Shi, P. Boireau, I. Vallee, X.L. Liu, X.L. Wang** Developmental profile of immune cells in mice infected with *Trichinella spiralis* during intestinal phase
HPI–2	**Justyna Bień, Witold Stefański, Anna Zawistowska–Deniziak, Katarzyna Wasyl, Bożena Moskwa** Immunomodulatory properties of various life stages of *Trichinella spiralis* and muscle larvae excretory–secretory products
HPI–3	**Francisco Bolás–Fernández, Luis Menchén Viso, Beatriz López–Cauce, Juan A. Rodríguez–Feo, Marta Puerto–Cantero, Juan José García–Rodríguez** Modification of the *Trichinella spiralis* intestinal settlement after antibiotic Treatment
HPI–4	**Jing Cui, Shuai Bing Zhang, Peng Jiang, Ruo Dan Liu, Shao Rong Long, Li Na Liu, Xi Zhang, Hui Jun Ren, Zhong Quan Wang** SiRNA–mediated silencing of Nudix hydrolase in *Trichinella spiralis* results in the reduction of larval infectivity
HPI–5	**Emília Dvorožňáková, Barbora Bucková, Zuzana Hurníková, Viera Revajová, Andrea Lauková** Effect of probiotic bacteria on phagocytosis and respiratory burst activity of blood polymorphonuclear leukocytes in mice infected with *Trichinella spiralis*
HPI–6	**Yuan Gu, Ximeng Sun, Jing Yang, Xiaohuan Wang, Xinping Zhu** Identification of Th2 epitope of paramyosin from Trichinella spiralis

Wednesday, 16th September 2015

13:00–14:00 hrs	**Host–Pathogen–Interaction and Immunology**
HPI–7	**Falduto Guido Hernán, Vila Cecilia Celeste, Saracino María Priscila, Gentilini María Virginia, Venturiello Stella Maris** Regulatory parameters of the lung immune response during the early phase of experimental trichinellosis
HPI–8	**Jana Ilgová, Lucie Škorpíková, Břetislav Koudela, Martin Kašný** Cysteine peptidase inhibitors of *Trichinella spiralis*
HPI–9	**Ahmad A. Othman, Dina M. Abo Raya, Dalia S. Ashour, Eman M. Saied, Ahmed A. El–Ebiary, Doaa. H. Zineldeen** Biochemical alterations of host environment can modulate experimental *Trichinella spiralis* infection
HPI–10	**Jolanta Piekarska, Michał Gorczykowski, Marianna Szczypka, Alicja Z. Kucharska** Modulation of lymphocyte populations by cornelian cherry (*Cornus mas L.*) active compounds in mice infected with *Trichinella spiralis*
HPI–12	**I. Symeonidou, S. Pappa, A. Kourelis, E. Karagouni, A. Frydas, A. Anogeianaki, M. Hatzistilianou** Application of microarrays to the analysis of Nitric Oxide pathway in monocytes of mice infected with *Trichinella spiralis*
HPI–13	**X. L. Wang, J. Liu, M.Y. Liu, X. Bai, S.M. Sun, X.P. Wu, Y. Wang, P. Boireau, H.N. Shi, X.L. Liu** Inhibitory effect on BALB/c nude mice bearing human H7402 solid tumor by administrated the A200711 protein from *Trichinella spiralis*
HPI–14	**Xinping Zhu, Jing Yang, Ximeng Sun, Yuan Gu, Wei Pan, Wei Zhu, Xi Zhao, Qing Sun, Jingjing Huang** Cloning and immunological identification of the 14–3–3 protein from *Trichinella spiralis*
HPI–15	**Zhong Quan Wang, Shao Rong Long, Ruo Dan Liu, Li Na Liu, Ling Ge Li, Peng Jiang, Xi Zhang, Hai Ning Shi, Jing Cui** Characterization and functional analysis of *Trichinella spiralis* Nudix hydrolase
13:00–14:00 hrs	**Detection**
DET–1	**Bao–Quan Fu, Nian–Zhang ZHANG, Wen–Yan GAI, Wen–Hui LI, Hong–Bin YAN, Zi–Gang QU, Jian–Min CUI** Prokaryotic expression and reactivity analysis of serine proteinase inhibitor gene of *Trichinella spiralis*
DET–2	**X. P. Wu, Z.J. Sun, X.L. Liu, X. Bai, X.L. Wang, B. Tang, B. Rosenthal, P. Boireau, J.X. Chen, X.N. Zhou, M.Y. Liu** Antibodies dynamics of mice infected with *Trichinella spiralis*
DET–3	**Alvin A. Gajadhar, Vladislav A. Lobanov** New strategies for improving the serodiagnosis of *Trichinella* infection in pigs
DET–4	**Jennifer Neumann, Sabine Reckinger, Karsten Nöckler, Anne Mayer–Scholl** Validation of the Trichin–L Antigen Test Kit for the detection of *Trichinella* larvae in meat products

Thursday, 17th September 2015

13:00–14:00 hrs	**Epidemiology**
EPI–1	**Pietro Badagliacca, D. Di Sabatino, G. Romeo, S. Salucci, M. Cipriani, N. Sulli N., F. Dall'Acqua, M. Ruggieri, D. Morelli** Endemic sylvatic trichinosis in Abruzzi region (Central Italy) and the epidemiological role of the wolf
EPI–2	**Justyna Bień, Aleksandra Cybulska, Aleksandra Kornacka, Mirosław Welc, Popiołek Marcin, Bożena Moskwa, Władysław Cabaj** Trichinellosis in wolves (Canis lupus) in Poland
EPI–3	**Ewa Bilska–Zając, Giuseppe La Rosa, Edoardo Pozio, Mirosław Różycki, Tomasz Cencek** Investigation on the genetic structure of *Trichinella spiralis* from pigs, rats and wild boar of Poland
EPI–4	**Břetislav Koudela, Jiří Harna, Martin Pijáček** Trichinellosis in wild boars in the Czech Republic
EPI–5	**D. Balić, Z. Krovina, G. Marucci, M. Benić, M. Agičić, M. Škrivanko** Trichinelosis in wild boar in Croatia (2010–2014)
EPI–6	**Fariña, F., M. Pasqualetti, Ercole, M., Cardillo, N., Rosa, A, Krivokapich, S. Ribicich** Evaluation of the infectivity and the persistence of *Trichinella patagoniensis* in a new host, the guinea pig
EPI–7	**W. Glawischnig, C. Schleicher, K. Schoepf** Current results of the assessment of the prevalence of *Trichinella* spp. in red foxes (Vulpes vulpes) in the Western Alpine regions of Austria
EPI–8	**W. Glawischnig, E. Vanek, A. Wunsch, H. Foetschl, K. Schoepf, F. Schmoll** First report of *Trichinella pseudospiralis* in Austrian wild boars (*Sus scrofa*)
EPI–9	**Zuzana Hurníková, Daniela Antolová, Martina Miterpáková, Nicole Březinová, Viktória Čabanová, Katarína Reiterová** Seroprevalence of *Trichinella* spp. in domestic dogs in Slovakia
EPI–10	**Zuzana Hurníková, Emília Dvorožňáková, Andrzej Zalewski, Marta Kołodziej–Sobocińska** *Trichinella* parasite in invasive American mink (*Neovison vison*) in Poland
EPI–11	**Age Kärssin, Liidia Häkkinen, Enel Niin, Katrin Peik, Annika Vilem, Pikka Jokelainen, Brian Lassen** *Trichinella* spp. in raccoon dogs (*Nyctereutes procynoides*) and red foxes (*Vulpes vulpes*) hunted in 2011–2012 in Estonia
EPI–12	**Dace Keidane, Anna Krūklīte, Kristīne Ganola** The research of *Trichinella* prevalence of wild boars in areas affected by hunting
EPI–13	**Muza Kirjušina, Zanda Segliņa, Gunita Deksne, Inese Jahundoviča, Eduards Bakasejevs, Giuseppe La Rosa, Edoardo Pozio** High prevalence of *Trichinella* spp. infection in carnivore mammals of Latvia

Thursday, 17th September 2015

13:00–14:00 hrs	**Epidemiology**
EPI–14	**L. Lider, O. Akibekov, A. Mayer–Scholl, K. Nöckler, M. Kuibagarov, S. Tokpan, Z. Suranshiyev, B. Ibrayev** *Trichinella* spp. in Northern Kazakhstan
EPI–15	**Bożena Moskwa, Aleksandra Cybulska, Aleksandra Kornacka, Justyna Bień, Władysław Cabaj** The occurrence of Trichinella spp. in respect to the gender of red foxes (Vulpes vulpes): preliminary results
EPI–16	**Bożena Moskwa, Aleksandra Cybulska, Aleksandra Kornacka, Justyna Bień, Marek Bogdaszewski, Żaneta Steiner, Artur Jabłoński, Władysław Cabaj** Wild boars meat as a potential source of human *Trichinella* cases in Poland
EPI–17	**Irina M. Odoyevskaya, Alexander V. Uspensky, Ivan V. Seriodkin, Lidia A. Bukina** The peculiarities of trichinellosis epidemiology in the Arctic territories of the Far Eastern Federal District of Russia
EPI–18	**Janez Posedi** *Trichinella* infection in fox (*Vulpes vulpes*) in Slovenia
EPI–19	**Edoardo Pozio, Muza Kirjušina, Eduards Bakasejevs, Patrizio Pezzotti** *Trichinella britovi* biomass in naturally infected pine martens (*Martes martes*)
EPI–20	**Milena Zivojinovic, Ljiljana Sofronic Milosavljevic, Jelena Cvetkovic, Sonja Radojicic, Budimir Plavsic, Ivan Dobrosavljevic, Zoran Kulisic** The most important risk factors for domestic and sylvatic cycle of *Trichinella* species identified in an endemic district of Serbia

Friday, 18th September 2015

13:00–14:00 hrs	**Human Trichinellosis and Treatment**
HUM–1	**Jean Dupouy–Camet** Trichinellosis and ancient mummies
HUM–2	**Cristina Dobrescu, Codruta Nemet, Mihaela Emandi, Carmen Zamfir** Clinical forms of manifestation of human trichinellosis in Braşov County, Romania, for a period of 30 years
HUM–3	**Bozena Moskwa, Daniela Antolová, Peter Jarčuška, Martin Janičko, Katarína Reiterová, Miroslava Škutová, Monika Halánová, Lenka Čechová, Lýdia Čisláková, HepaMeta team** Seropositivity to *Trichinella* spp. in Roma population from segregated settlements and in non–Roma population of Eastern Slovakia
HUM–4	**Sasa Vasilev, Andjelka Korovljev, Mirko Doroslovac, Milovan Djordjevic, Ivana Trailovic, Marija Devic, Ljiljana Sofronic–Milosavljevic** Trichinellosis in Serbia, evidence on long lasting antibody presence: pilot study
HUM–5	**F. Bruschi, S. Piaggi, C. Bianchi, C. D'Amato, B. Castagna, A. Paolicchi, B. Pinto** MMP–9 and 2 in human trichinellosis

Friday, 18th September 2015

13:00–14:00 hrs	**Legislation and Control**
LEG–1	**Gianluca Marucci, Daniele Tonanzi, Isabelle Vallee, Karsten Nöckler, Tamas Sreter, Jiri Harna, Edoardo Pozio** Validation of the PrioCHECK® *Trichinella* AAD KIT for the detection of *Trichinella* infections in pigs
LEG–2	**Gianluca Marucci, Daniele Tonanzi, Simona Cherchi, Fabio Galati, Antonino Bella, Edoardo Pozio** Proficiency testing to detect *Trichinella* larvae in meat: Report of nine years of activity at the European Union Reference Laboratory for Parasites
LEG–3	**G. Makrutzki, K. Riehn, A. Hamedy, M. Koethe, E. Lücker** Sedimentation funnel as a new source of error in official *Trichinella* examination
LEG–4	**G. Makrutzki, A. Hamedy, S. Dolle, S. Birka, K. Riehn, E. Lücker** A current status of evidence on *Alaria* spp. mesocercariae in game
LEG–5	**Edoardo Pozio, Ifor Owen, Maria Angeles Gomez Morales** Cooking methods and infection with *Trichinella papuae* in Papua New Guinea
LEG–6	**Miroslaw Różycki, Ewa Bilska–Zając, Ewa Chmurzyńska, Jacek Karamon, Tomasz Cencek** Validation of digestion assay based on results of proficiency comparison results 2007–2014 in Poland
LEG–7	**X.P. Wu, D. Wang, X. Bai, X.L. Liu, X.L. Wang, B. Tang, Z.J. Sun, B. Rosenthal, P. Boireau, J.X. Chen, X.N. Zhou, M.Y. Liu** The study of optimized conditions of artificial digestion method for inspection of *Trichinella spp.*
LEG–8	**Stefanie Willen** Trichinellosis in Baden–Württemberg
LEG–9	**D. Schlichting, M. Greiner, A. Mayer–Scholl, A. Käsbohrer, K. Nöckler, C. Müller–Graf** Monitoring of *Trichinella* in pigs – sample size estimation

2 Abstracts for keynotes and oral presentations

2.1 Phylogeny, Genomics and Proteomics

Keynote

Phylogeny and comparative population biology of *Trichinella* spp.

Benjamin Rosenthal

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The diversity and evolutionary history of species in the genus *Trichinella* are becoming clearer now that high–throughput sequencing has begun to be applied to the mitochondrial and nuclear genomes of each known member of the genus.

Estimates of phylogenetic relationships available for a decade have been strengthened and, in certain cases revised, by new data, but mysteries persist concerning the evidently long interval separating the genus from any other known group of nematodes. Although stable phylogenetic estimates provide an essential framework for understanding the forces that have shaped the historical diversification and dissemination of these parasites, opportunities still exist for innovating informative and cost–effective ways to apply molecular population genetics to clarify epidemiological processes and trace outbreaks, especially for *T. spiralis* which (outside of Asia), exhibits strikingly limited variability among isolates.

By helping to identify natural hybrids, molecular variation has recently been employed in ways that suggest the possibility of gene flow between recognized taxa, but available evidence continues to suggest stability in distinctions that have been recognized among taxa in this genus. The purpose of this talk will be to review recent advances, point out lingering needs, and provoke future progress in this realm.

Phylogeny, Genomics and Proteomics

Keynote

Genomes of all members of the *Trichinella* complex: phylogenomic and biogeographic reconstruction

Pasi K. Korhonen[1], Edoardo Pozio[2], Giuseppe La Rosa[2], Bill Chang[1,3], Anson V. Koehler[1], Eric P. Hoberg[4], Peter R. Boag[5], Patrick Tan[6,7], Aaron R. Jex[1], Andreas Hofmann[1,8], Paul W. Sternberg[9], Neil D. Young[1], Robin B. Gasser[1]

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Trichinellosis is a parasitic disease of humans caused by nematodes of the genus *Trichinella*, and represents a globally important zoonosis. *Trichinella* has a wide geographical range, comprises at least nine recognised species and three genotypes, with extensive biodiversity and varying degrees of host usage (mainly mammals, birds and/or reptiles) and dispersal.

This biodiversity of *Trichinella* is reflected in substantial genetic ecological variation, and there has been controversy surrounding systematic relationships of members of this genus due to very limited genomic data. Here, we sequenced and assembled 16 draft nuclear genomes (48–59 Mb) and corresponding transcriptomes of all currently recognised *Trichinella* taxa.

We reconstructed the phylogeny and biogeography of all taxa (including five distinct geographical populations of one taxon) using thousands of orthologous gene sequences, and then explored parasite–host relationships at the molecular level. We show that *Trichinella* taxa diverged from their most recent common ancestor ~ 21 million years ago (mya), with diversifications within distinct (encapsulated and non–encapsulated) clades commencing ~10–7 mya, consistent with a previous prediction using small datasets. The significantly higher GC content in the genomes and coding regions of encapsulated compared with non–encapsulated taxa suggests that these two *Trichinella* clades might have adapted differently to varying environmental stresses (e.g., temperature), host immune attack and/or body temperatures (reptiles vs. birds and mammals). We undertook transcriptomic comparisons to show that some excretory/secretory (ES) molecules, including serine peptidase–like molecules, are likely to be central to the parasite–host interplay, the modulation of host attack and also host affiliations.

The genomic and transcriptomic resources established here for all described *Trichinella* taxa will underpin a wide range of future systematic and population genetic studies as well as the development of new diagnostic and intervention tools.

Phylogeny, Genomics and Proteomics

Interspecies recombination between *Trichinella spiralis* and *Trichinella britovi* occurs under natural conditions

Frits Franssen[1], Ewa Bilska–Zając[2], Gunita Deksne[3], Hein Sprong[1], Edoardo Pozio[4], Benjamin Rosenthal[5], Mirek Rozycki[2], Joke van der Giessen[1]

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To date, 12 taxa are recognised in the genus *Trichinella*, of which four are circulating in Europe (*Trichinella spiralis, Trichinella nativa, Trichinella britovi and Trichinella pseudospiralis*). *T. spiralis* and *T. britovi* circulate in European wildlife and occur simultaneously in the same host species. The possibility of hybrid formation between *T. britovi* and *T. spiralis* has hardly been addressed and so far, results of experimental hybridisation attempts between *T. britovi* and *T. spiralis* are inconclusive.

The aim of the present study was to analyse molecular polymorphisms of single *T. spiralis* and *T. britovi* (ML) from natural infections based on the nuclear 5S ribosomal RNA intergenic region (5S rDNA) and mitochondrial cytochrome c oxidase 1 (CO1) gene sequences.

Trichinella spp. larvae were collected from 35 wild boar and 6 foxes of Poland and from 1 wild boar and 30 red foxes of Latvia, during routine detection of *Trichinella* spp. parasites by artificial digestion. Six haplotypes of the 5S rDNA and 14 of the CO1 gene were demonstrated in 89 individual *T. britovi* ML from Latvia and Poland. In contrast, only two haplotypes were observed at both 5S rDNA and CO1 of 57 individual *T. spiralis* ML from Polish wild boar and red foxes, which can be explained by the relatively recent introduction of *T. spiralis* into Europe, estimated at 1000 years before the present (BP), in contrast to the expansion of *T. britovi* into Europe estimated at 15–20 million years BP.

Joint evaluation of 5S rDNA and CO1 sequence information of individual *Trichinella* ML revealed that out of 154 tested individual *Trichinella* ML, eight larvae were hybrids between *T. britovi* and *T. spiralis*, which were isolated from four wild boars and two red foxes from Poland. All hybrids were combinations of the most prevalent haplotypes, which implies that possibly more hybrid haplotype combinations could exist, for which analysis of a far greater sample size is needed to demonstrate those combinations.

Infection experiments with pairs of single male and female larvae of either *Trichinella* species in the present study showed that hybridisation comes with apparent cost of reduced offspring in F1, which could drive the F2 progeny towards segregation of rDNA alleles, resulting in the transmission of one matrilineal haplotype to two distinct genomic backgrounds. This cost appears higher for male *T. spiralis* with female *T. britovi* hybridisation than for the other direction (female *T. spiralis* with male *T. britovi*).

Phylogeny, Genomics and Proteomics

Proteomic analysis of differentially expressed proteins from muscle larvae and pre–adult stages of *Trichinella spiralis*

G. Ortega–Pierres[1], L.E. Grijalva–Contreras[1], R. Fonseca–Liñán[1], Guillermo Mendoza[2], V. Flores–López, R.M. Bermúdez–Cruz

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Trichinella spiralis infects a variety of mammals including humans causing trichinellosis. An important aspect in the study of this parasite is the development of prophylactic methods for the control of the infections caused by this nematode. In this context, antigens, particularly from *T. spiralis* muscle larva (ML), have been used to induce protection in rodent models. Among these TSL–1 antigens in combination with adjuvants have induced partial protection against *Trichinella* infection. Thus, it is necessary to identify components of other stages of the parasite that may increase protection in the host.

In this study antigens expressed by ML and pre–adults (PA) of *T. spiralis* collected from infected rats at 6, 18 and 30 pi were identified using Proteomics and mass spectrometry analysis.

The results showed 45 predominant and differentially expressed proteins between the ML and PA with MW of 13–417 kDa and isoelectric point of pH 4.2–8.8. Further, a differential recognition of parasite proteins from ML and PA by antibodies in the intestinal fluid from infected mice collected 12 days pi. was observed. In this assay, among others, a band of 47kDa was recognized in all stages of maturation and its recognition decreases as the parasite develops. Likewise 2 bands of approximately 27– 30 kDa were detected in PA from 6H and 18H while a lower recognition of these components in ML and 30 H PA was observed. *In silico* analyses revealed that, the identified proteins are related to a great diversity of functions: metabolic pathways, cytoskeleton, translation, endoplasmic reticulum processing, protein folding, signal transduction, and interestingly proteases, as well as to redox–related proteins.

Our findings suggest that in spite of the major shifts in the protein profiles at different development stages, functions such as proteolysis and redox potential are relevant for this parasitic nematode. Prediction of MHC class I and class II epitopes in selected proteins showed that most of them have immunogenic peptides that constitute potential B and T–cell epitopes suitable as vaccine candidates. Peptide–based vaccines may offer means for safe and precisely directed immune intervention therefore selected peptides from differentially expressed proteins by developmental stages of this parasite may be useful to induce a better protection against *T. spiralis*. These proteins may also be considered as candidates for an early diagnosis of trichinellosis.

Phylogeny, Genomics and Proteomics

Trichinella spiralis; adaptation and parasitism

Dante Zarlenga[1], Zhengyuan Wang[2], Makedonka Mitreva[2]

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Parasitism among nematodes has occurred in multiple, independent events. Deciphering processes that drive species diversity and adaptation are keys to understanding parasitism and advancing control strategies. Studies have been put forth on morphological and physiological aspects of parasitism and adaptation in nematodes; however, data is now becoming available to investigate adaptation, host switching and parasitism at the genomic level.

Herein we explore the association between changes in protein families and domains over the course of metazoan evolution and the relationship between these changes and the ability and/or result of nematodes adapting to their environments. Data are consistent with gene loss occurring in conjunction with nematode specialization resulting from worms acclimating to well–defined, environmental niches. Further, we observed evidence for independent, lateral gene transfer events involving conserved genes that may have played a role in the evolution of nematode parasitism.

Of special interest is the existence of the protein, cyanase in a select few parasitic nematodes, including those belonging to the clade I parasites, *Trichinella and Trichuri,* and that appears to have bacterial or fungal origins. Cyanase was also found present in more disparate clade III parasitic nematodes but not in the genome of another Dorylaimia, *Romanomermis culicivorax.* Acquisition of the gene from nematophagous fungi may provide evidence of independent acquisition in clade III nematodes rather than ancestral acquisition among the Nematoda followed by selective gene loss over evolutionary time.

Phylogeny, Genomics and Proteomics

The comparison of functional molecules from excretory–secretory products of two *Trichinella* species

Martin Kašný[1,2], Lucie Škorpíková[2], Jana Ilgová[1], Břetislav Koudela[3,4], Milan Gelnar[2], David Potěšil[5,6], Zbyněk Zdráhal[5,6], Peter Thompson[7], Dante Zarlenga[7]

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Trichinellosis remains an important worldwide zoonotic disease. Today, however, infections result primarily from sylvatic hosts, free range farming and the raising of “backyard” pigs in highly endemic areas. Currently, the genus has been partitioned into 9 species with at least 3 other genotypes whose taxonomic status remains in flux. Our study focused on characterizing proteins from muscle larvae of two *Trichinella* species representing the encapsulated and non–encapsulated clades; *Trichinella spiralis* which parasitizes mainly pigs and wild boars and predominates within the domestic cycle, and *T. pseudospiralis* which infects mainly avian hosts, respectively.

We used High–Performance Liquid Chromatography (UltiMate® 3000 RSLCnano LC) followed by Mass Spectrometry (Orbitrap Elite) in combination of transcriptomic analysis (Illumina MiSeq) to reveal and compare the spectrum of proteins present in excretory–secretory (ES) products and those which are actively transcribed by these model organisms. Several of important functional protein compounds were observed and their key role in host–parasite cross–talking was estimated. Among the ES products that were identified, 347 were found in *T. spiralis* and 508 in *T. pseudospiralis* L1 larvae. Typical among the antigens were the 53 kDa and 43 kDa immunodominant glycoproteins. We also identified biologically active proteins such as cysteine peptidases i.e. cathepsin F, B, and their inhibitors the cystatins. Some of these molecules have been cloned (cathepsin F, B, 53 kDa protein, 43 kDa glycoprotein, cystatins) and expressed (53 kDa protein, 43 kDa glycoprotein, cystatins). A transcriptomic analysis of cDNA from both organisms (Illumina MiSeq) was performed in order to reveal which genes are actively transcribed in the L1 and their relative expression levels.

In total, 71,916,312/71,559,838 paired ends reads were generated for *T. spiralis/T. pseudospiralis*, respectively. The assembly of trimmed and filtered sequences had the best parameters after processing the data in SOAPaligner/SOAP2. Among the 12,464 *T. spiralis* genes´sequences identified, 5515 were mapped back to the genome; however, only 87 of 6534 genes from *T. pseudospiralis* (coverage 90–100 %) could be identified.

Phylogeny, Genomics and Proteomics

Immunoproteomic analysis of the excretory–secretory products of the *Trichinella pseudospiralis* adult and newborn larvae

X.L. Liu[1,] Y. Wang[1§], M.Y. Liu[1,6], X. Bai[1§], H. Gao[1], X.P. Wu[2], T.T. Li[1], H.N. Shi[3], P. Boireau[4], B. Rosenthal[5], X.L. Wang[1]

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Compared with *Trichinella spiralis* infection, *Trichinella pseudospiralis* causes considerably less inflammation in the intestine and muscle of host, suggesting its stronger immunomodulation ability. Although the excretory–secretory (ES) products are thought involved in immunosuppression, the molecules mechanisms remain largely unknown.

In this study, the ES proteins of *T. pseudospiralis* adult (AD) and newborn larvae (NBL) were analyzed by two–dimensional gel electrophoresis (2–D) coupled with immunoblotting. The immunoreactive spots recognized by early infection sera from swine infected with *T. pseudospiralis* were characterized by liquid chromatography–tandem mass spectrometry (LC–MS/MS). A total of approximately 400 spots were separated with isoelectric point (pI) varying from 4 to7 and molecular weight from 10 to 130 kDa.

More than 60 protein spots were recognized with sera from infected swine at 26 and 60 days post infection in the ES products of 3–day–old adult worms (AD3). Approximately 80 protein spots were identified in the ES products of 6–day–old adult worms/newborn larvae (AD6+NBL) by western blot analysis. Several proteases including serine–type proteases, metalloproteinases, 5'–nucleotidase and heat shock protein 70 showed high immunogenicity. These proteins might participate in the process of immune regulation.

[§]These authors contributed equally to the work.

Phylogeny, Genomics and Proteomics

Assortative mating limits hybridization between two lineages of freeze–resistant *Trichinella*

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Hybridization between two closely related but distinct genetic lineages may lead to homogenization of the two lineages with potentially novel phenotypes, or selective pressure to avoid hybridization if the two lineages are truly distinct.

Trichinella nativa and *Trichinella* T6 are zoonotic nematode parasites which can be distinguished genetically despite occasional hybridization. Here, using an experimental murine model, we attempt to determine whether these taxa hybridize freely or avoid mating with each other when sizeable numbers of each lineage are allowed to coinfect a host. The population of worms was followed for five generations, and individuals from the F1, F2, and F5 generations were genotyped at 3 microsatellite loci and one mitochondrial locus capable of distinguishing *T. nativa* from T6 genotypes. Among larvae in the F1 generation, offspring of every possible mating were encountered.

Most larvae (69 %) derived from *T. nativa* x *T. nativa* matings, while 17 % of offspring were the product of T6 x T6 matings, and only 14 % were hybrid offspring of *T. nativa* x T6 crosses, differing markedly from random mating expectations. Over five generations, T6 alleles gradually decreased in frequency while *T. nativa* alleles became predominant, accounting for greater than 90 % of all alleles.

In this experimental model, *T. nativa* and *Trichinella* T6 tend to mate within lineage, and *T. nativa* has higher reproductive fitness perhaps because of higher fecundity. This assortative mating would limit gene flow between these two lineages in a natural setting, serving as a barrier to their homogenization and promoting their persistence as distinct and separate entities.

2.2 Biology, Host–Pathogen–Interaction and Immunology

Keynote

The biology of *Trichinella*: from genes to genomes

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The nematode phylum can be divided into two major classes, the Chromadorea and the Enoplea, of which the latter class has been more extensively studied largely because it includes the free–living model nematode *Caenorhabditis elegans*. In fact, much of our understanding of nematode biology comes from the study of *C. elegans*.

However, it is increasingly apparent that the study of the nematodes of the Chromadorea class alone gives an incomplete picture of nematode evolution and biology. *Trichinella spiralis* belongs to the Enoplea and is of phylogenetic importance and interest due to its position near the base of the nematode phylum. It remains one of the few basal nematodes for which there is available genome data and is thus uniquely placed to provide information on the ancestral nematode and the evolution of the nematodes.

Our analysis of *T. spiralis* along with other basal nematodes, *Trichuris muris* (parasitic) and *Prionchulus punctatus* (free–living) is providing additional insights into the biology of basal nematodes. For example, signalling pathways either missing or altered in *C. elegans* have been identified in these species. Of recent interest are our studies on spliced–leader (SL) *trans*–splicing and the organisation of genes into operons in the basal nematodes in general and *T. spiralis* in particular. Operons are clusters of genes that are co–transcribed to produce polycistronic pre–mRNAs and are found in a wide–range of eukaryotic groups, although their distribution is sporadic. They are present in *C. elegans* and other members of the Chromadorea, where they have been well–studied and where the resolution of the polycistronic mRNA occurs by SL *trans*–splicing. We have data identifying the first putative operons in *T. spiralis* and *T. muris*, along with evidence that the mRNAs produced are *trans*–spliced.

We find that the orthologues of genes located in operons in *T. spiralis* are more likely to also be in operons in *C. elegans*, consistent with models of operon evolution, and have identified putative operons conserved between the two species. Our data suggest that operons and SL *trans*–splicing are common features of all nematodes and predate the radiation of the nematode phylum. Within the Chromadorea the conserved spliced leader SL1 is found in all species, whereas a second specialized SL2 is found only in some species where it is associated with *trans*–splicing of downstream genes in operons. Previously, we have shown that *T. spiralis* and *T. pseudospiralis* have unusually diverse SLs that do not readily correspond to those SLs found within Chromadorea.

Surprisingly, when we extended our analysis to the other basal nematodes *P. punctatus* and T*. muris* we found evidence that they possessed SLs resembling the specialised SL2 found in *C. elegans* and other closely related nematodes. This suggested that the diverse SL complement in *Trichinella* species is likely to be a derived rather than ancestral trait. We will now show evidence for functional specialisation of the *Trichinella* SLs and data suggesting

that a subset of the SLs (Tsp–SL2, Tsp–SL10 and Tsp–SL12) are involved in the resolution of polycistronic mRNAs and are almost exclusively employed in trans–splicing to the downstream genes of operons.

Biology, Host–Pathogen–Interaction and Immunology

Keynote

Host pathogen interaction: antigenic shift in *Trichinella* development

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The life cycle of *T. spiralis* is completed within a single host species and infection starts with the consumption of infective muscle larvae (ML) and digestion of the protective capsule within the host stomach. The invasive stage is equal to the last stage in the same host, meaning that larvae antigens are exposed immediately to the new host. To overcome this apparent disadvantage, larvae undergo four fast molts in intestinal epithelial cells and eventually develop into sexually mature adults (Ad) approximately 2–3 days post infection (pi). Freshly released newborn larvae (NBL) are carried to host tissues by blood flow and invade new host cells. The NBL penetrate striated muscle cells and undergo developmental changes in few days. Larvae that are older than 14 days can be infective to subsequent potential hosts and may remain viable for the entire life span of the host.

To date, little is known about the molecular mechanisms that are involved in parasite development and survival within the cytoplasm of the host cell. Identification of stage–specific genes/proteins is important for the elucidation of these mechanisms. ML, Ad and NBL are three major stages in the life cycle of *T. spiralis* that exhibit distinct antigenicity (antigenic shift), indicating differential regulation of many parasite proteins. The first developmentally regulated antigens fully characterized in the ML were the stage–specific TSL–1 antigens identified by monoclonal antibodies. TSL1 is composed of at least 6 different glycoproteins carrying the same immunodominant carbohydrate: a beta tyvelose capping antennae structure (present on the surface of the cuticle and in alpha granule of stichosome). However, data indicated that this sugar is also shared by eggs of other nematodes and has no protective role. Several TSL1 glycoproteins were cloned and expressed in various systems, p49, gp53. An epitope mapping performed on different antigens (gp 53). In brief, studies with various monoclonal antibodies indicated that each *Trichinella* stage is composed of a cuticle exhibiting a bulk of stage specific antigens.

In an attempt to identify stage – specific genes and antigens of *T. spiralis*, subtracted cDNA libraries of NBL, Ad3 and Ad5 were constructed respectively, using a suppression subtractive hybridization (SSH) technique and screening of cDNA libraries with various sera from pig experimentally infected with *Trichinella spiralis* or *Trichinella britovi*. A number of stage – specific cDNAs derived from NBL, Ad3 and Ad5 were identified confirming the antigenic drift during *Trichinella* development. Several genes were identified as NBL stage–specific**,** including one member of the *T. spiralis* gene family encoding glutamic acid rich proteins, two genes encoding serine proteases, two closely related genes encoding proteins that are members of a deoxyribonuclease II (DNase II)–like family and one nucleotidic sequence with no similarity to known genes.

The twin genes encoding DNaseII (*DnaseII1*$^{Ts/NBL}$ and *DnaseII2*$^{Ts/NBL}$) have a high percentage of identity in their amino acid (aa) sequence (89.6 %), and their predicted aa sequences exhibited a N–terminal signal peptide, a potential helix–loop–helix motif and the conserved domains of DNase II. Four stage–specific clones encoding homologues of retinoid X

receptor, caveolin, C2H2 type zinc finger protein and a putative protein with no homology to known sequences were obtained from 3–day–old adult worms.

The caveolin–1 gene (*Cav*^{Ts}) was characterized and identified as an adult–specific antigen. *Cav*^{Ts} is gradually accumulated only on the ova surface reaching a maximum at 3 days pi, and decreasing during newborn larva (NBL) development. Another target (*AdTs1*) was analysed in a 3–day–old adult subtractive cDNA library. The selected gene encodes a protein with two putative zinc finger domains. Interestingly, some strong similarities were found between the AdTs1 protein, nuclear hormone receptors of mammals and a molting marker of *C. elegans*. Additional immunodominant stage specific antigens were identified at the very early development of *Trichinella* in the intestinal tract between 14hours pi and 48hours pi. A summary of different immunodominant epitopes published by different authors will be done to indicate future strategy to combine them for diagnostic purpose or the design of recombinant vaccines.

Biology, Host–Pathogen–Interaction and Immunology

Individual components of *Trichinella spiralis* excretory–secretory muscle larvae antigens – the role in shaping of the immune response

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Trichinella spiralis muscle larvae excretory–secretory antigens (ES L1) are most likely responsible for the induction of immune response during muscle phase of the infection. However, there are limited data about the individual components of ES L1 which may contribute to immune system activation resulting in a Th2/anti–inflammatory immune response. It has been demonstrated that either human or animal immune sera recognize three components of ES L1 antigens that bear the immunodominant epitope characteristic for the genus *Trichinella* – 45, 49 and 53 kDa glycoproteins. Based on that observation, the aim of this study was to investigate the way in which two antigen preparations: the above mentioned triplet from ES L1 antigens and 53 kDa recombinant protein, affect the induction and polarization of immune response.

Namely, the impact of these ES L1 antigenic components on dendritic cells (DCs) phenotype and functional characteristics, triggering signal pathways and subsequent T cell polarization were explored. Dendritic cells stimulated with either of these antigens obtained typical morphological characteristics of semi–matured cells which correspond with those observed with complete ES L1 antigens. Functional characterization of these cells showed that investigated ES L1 antigenic components, as well as ES L1 itself, induced the increased production of IL–10 while the concentrations of IL–12p70 remained unchanged compared to the levels obtained with non–stimulated cells.

In vitro priming of naïve T cells with DCs stimulated with each of analyzed ES L1 antigen preparations emphasized the capacity of these DCs to induce significantly increased proliferation of naïve T cells and polarization of immune response towards Th2/anti–inflammatory type, while only DCs stimulated with 53 kDa recombinant protein showed the potential to activate the regulatory arm of the response. In accordance to the observed cytokine profile, it was shown that both preparations induce transient activation of ERK1/2 and week activation of p38. Although both ES L1 components activate MAPK signaling pathways, their potential for this activity is significantly lower compared to the one exhibited by complete ES L1.

Results obtained in this study strongly indicate that both ES L1 triplet and 53 kDa recombinant protein contribute to the effect that complete ES L1 exhibits in triggering and polarization of the immune response.

Biology, Host–Pathogen–Interaction and Immunology

The presence of galectin–1–like molecules in *Trichinella spiralis* muscle larvae excretory–secretory antigens

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During the infection with *Trichinella spiralis,* excretory–secretory muscle larvae (ES L1) antigens are responsible for creating anti–inflammatory environment dominated by Th2 and regulatory responses. This complex mixture of various molecules, which acts via antigen presenting cells to induce polarization of immune response, contain proteins whose function was determined mostly in accordance to their structural and sequence similarity with proteins from another species with known function.

We have analyzed ES L1 antigens for the presence of galectin–1 (Gal–1) – like proteins, since Gal–1 possess immunomodulatory functions important for the resolution of inflammatory responses. Galectins are highly conserved family of glycan–binding proteins with widespread distribution in the animal kingdom. Immunohistological analyses of *T. spiralis* muscle larvae with anti–Gal–1 antibodies produced staining in the subcuticular region and in the stichosome. Western–blot of ES L1 antigens, performed to determine which parasitic component reacts with anti–Gal–1 antibodies, revealed existence of several molecules, ranging between 40 and 60 kDa and some with higher molecular masses that presumably have structural similarities with Gal–1.

Affinity chromatography of ES L1 antigens on immobilized lactose (Gal–1 specific sugar), under reducing conditions, resulted in the isolation of specific fractions, bound on the basis of their lectin activity. Interaction of isolated molecules with anti–Gal–1 antibodies confirmed their structural similarity with Gal–1. Functional properties of isolated Gal–1–like molecules in terms of induction and polarization of the immune response were investigated *in vitro* on dendritic cells (DCs). Phenotype and functional characteristics of DCs stimulated with Gal–1–like molecules corresponded to those obtained with ES L1 i.e. they acquired the same semi–matured status and cytokine production.

Priming of naïve T cells with such DCs indicated the potential of these cells to drive immune response towards mixed Th1/Th2 type, accompanied by activation of regulatory mechanisms. Data obtained in this study suggest that *T. spiralis* ES L1 antigens comprise Gal–1–like molecules which probably participate in immunomodulatory functions of ES–L1.

Biology, Host–Pathogen–Interaction and Immunology

The ultrastructural analysis of pulmonary oedema formation in BALB/c mice infected with *Trichinella spiralis*

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Pulmonary oedema (PE) is fluid accumulation in the lungs, which collects in air sacs and may cause respiratory failure. PE is an important contributor to the pathology of *Trichinella spiralis.* This nematode exhibits a tissue–migratory larval stage, which pass through the lung microvascular system on their way to the skeletal muscles. Clinical manifestations of *T. spiralis* infection commonly include Löffler syndrome, bronchitis and abdominal pain or diarrhea associated with peripheral blood eosinophilia.

The purpose of this report was to describe the reasons of pulmonary oedema formation in the experimental *Trichinella spiralis* invasion. The immunocompetent mice (BALB/c) were infected with high number (800 L1) of *T. spiralis* larvae to induce pulmonary oedema.

The lung tissue samples were taken under ANSESthesia and were fixed with a mixture of 2 % paraformaldehyde and 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 20 hrs. Tissue sections were additionally postfixed in 1 % OsO_4 and 0.8 % K_4FeCN_6. Ultrathin sections were examined using a JEM 1,200 EX transmission electron microscope.

Our ultrastructural studies demonstrated that the tissue–migratory *T. spiralis* larval stage evoked mainly destruction of epithelial cells type I and disintegration of lamellar bodies of epithelial cells type II. We often observed eosinophils, lymphocyts and monocyts in the lumen of capillary vessels. At the same time in many capillaries thrombocyte aggregations were also recognized. In the pulmonary interstitium fibroblasts were associated with collagen fibers.

Alveolar macrophages were located in many pulmonary alveoli. We observed fluid in many pulmonary alveoli, which contained fine fibrous protein material. Unsealed junctions between capillary endothelial cells and epithelial cells were also present**.** This could facilitate the easy migration of the blood components to the pulmonary alveolus. This deficiency of type II pulmonary cells reduces the production of surfactant proteins**.** The surfactant elements, which form an extracellular alveolar lining layer, cannot stabilize alveolar surface tension and hinder gas exchange.

We should have emphasized that pulmonary oedema formation could be a result of toxic activity of *T. spiralis* antigens. It could be evoked by mechanical damage in the lung parenchyma during *T. spiralis* larvae passage through the lung. The consequence is incorrect function of air wall, dysfunction of surfactant and generalized pulmonary deficiency.

Biology, Host–Pathogen–Interaction and Immunology

T cell clones in human trichinellosis: Th2 polarization

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The cellular immune response to *Trichinella* is well known in rodents, in which a stable Th2 response is maintained along the infection after a short CD4 Th1 reaction. Information on the cellular immune response in humans in the course of trichinellosis is limited to the muscle phase in patients infected by *Trichinella spiralis* and *Trichinella britovi*, in which a type 2 cytokine pattern was observed. The aim of this study was to generate and isolate T cell clones specific to *T. britovi* antigens from patients with trichinellosis.

Peripheral blood mononuclear cells were collected three months post infection from 5 (3 males, median age 42.6 yrs, range 36–64) patients involved in a trichinellosis outbreak caused by *T. britovi*, which occurred in Tuscany (Italy) in November–December 2012. Informed consent was obtained from all the studied patients. All the enrolled patients were positive for *Trichinella*–specific IgG by western blot. T cell clones were obtained by standard procedure after stimulation with excretory/secretory antigens obtained from *T. spiralis* muscle larvae (TsES). Supernatants were collected and analyzed for interferon (IFN)– γ, tumor necrosis factor (TNF)–α, and IL–4. Supernatants showing IFN–γ, IL–4, and TNF–α levels 5 times the SD over the mean levels of control supernatants, derived from irradiated feeder cells alone, were regarded as positive. CD4+ T–cell clones able to produce IFN–γ, but not IL–4, were categorized as Th1; CD4+ T cell clones able to produce IL–4, but not IFN–γ, were categorized as Th2; CD4+ T cell clones producing both IFN–γ and IL–4 were categorized as Th0. A total number of 284 CD4+ and 42 CD8+ T–cell clones were obtained from the TsES–specific T cell lines. CD4+ and CD8+ T–cell clones from each patient were assayed for proliferation in response to medium, TsES, and PHA. All T–cell clones proliferated in response to mitogen stimulation.

Out of 284 CD4+ and 42 CD8+ T–cell clones, 135 (47 %) and 11 (26 %) generated from TsES–specific T–cell lines proliferated significantly to TsES, respectively. In the series of 135 TsES–specific CD4+ clones, 68 (51 %) expressed a Th2 profile, 41 (30 %) were Th0, and 26 (19 %) were Th1 clones. In the series of 11 TsES–specific CD8+ clones, 2 (18 %) were Th2, 5 (45 %) Th0 and 4 Th1 (36 %).

In conclusion, in humans as in rodents, the cellular immune response is Th2 polarized during the course of *Trichinella* infection.

Biology, Host–Pathogen–Interaction and Immunology

The inflammation induced by *N*–acetyl–glucosamine and glucosamine supports *T. spiralis* infection

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T. spiralis antigens may modulate dendritic cells activation and when incomplete, finally results in Th2 mediated immune response. Also pro–inflammatory activity of macrophages is inhibited by the nematode antigens and CD4+ cells play a key role in the resolution of *T. spiralis* infection. The nematode produces specific CD4+ T–cell response and develops Th2 driven transient inflammation in the small intestine what resulting in nematode expulsion via mast cell dependent process. However the full mechanism controlling enteritis and the precise effects on immunity to intestinal infection require study.

We used *Trichinella spiralis,* the nematode parasite which transiently inhabits the small intestine before migrating to skeletal muscle. The infection provides a useful biphasic model to study the processes controlling enteric and peripheral inflammation. Mice infected with *T. spiralis* are convenient to study the factors which alter protection during infection. We assume that enhancing activity of cells entangled in the innate immunity will support protection against *T. spiralis* infection. To provoke inflammatory response in the enteral phase of infection we used chitosan, a polymer – deacetylated derivative of chitin, as a source of N–acetyl–glucosamine and glucosamine chains for activation of cells in the peritoneal cavity. *T. spiralis* larvae express also these carbohydrate moieties which are immunogenic, what was confirmed by lectins and IgA. Chitosan given intraperitoneally before and during enteral phase of the infection induced inflammation that reduced the protection; greater number of adults (40 %) in the intestine and larvae (twice) in the muscle phase of infection were observed.

Chitosan induced inflammation on day 5th post *T. spiralis* infection and resulted in reduced number of resident macrophages together with greater number of eosinophils and huge infiltration with monocytes and neutrophils also in the blood. Later 30 days post infection response of cells reduced, however with enhanced macrophage accumulation and MCP–1, IL–10 and IL–6 production typical for tissue regeneration.

The level of macrophage/monocyte receptor expression e.g. MHC II, CD80, CD86, CD14, MR, Dectin1 and CD23 markedly dropped 5 days post infection along of concurrent peritonitis. Cytokine production was also changed upon chitosan and MCP–1, IL–12 with elevated TGF–beta, IL–10 and IL–6 mirrored unresolved profile of the immune induction. Therefore the keeping cells in that state of confusing immune activation promoted infection. Cell activation by glucosamine residue resulted in hesitant response of the innate immunity and supporting of *T. spiralis* infection in mice.

Biology, Host–Pathogen–Interaction and Immunology

Molecular cloning and characterization of a cathepsin–F–like protease from *Trichinella spiralis*

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Cathepsin F is a cysteine protease that is a major virulence factor in parasitic helminth, and it may be a potential anti–helminth drug target and vaccine antigen. In this study, the cDNA encoding a putative cathepsin F–like peptidase in *Trichinella spiralis*, TsCF1, was obtained and its biochemical characterization and expression profile were analyzed.

Results demonstrated that the TsCF1 gene encodes a protein of 366 aa with the theoretical molecular weight of 41.9 ku and isoelectric point of 7.46. The signal peptide sequence of TsCF1 located between amino acids 1 and 19 and 3 putative N–glycosylation sites were evaluated. The cysteine protease conserved active site of Cys173, His309 and Asn333 were identified and cathepsin F specific motif ERFNAQ like KLFNAQ sequence was revealed in the propeptide of TsCF1. Homology analysis revealed a higher than 40 % identity with other cathepsin F from parasitic helminth and phylogenetic analysis indicated that TsCF1 is located in a different clan with trematode cathepsin Fs. RT–PCR revealed TsCF1 to be expressed in muscle larvae, newborn larvae and adult stages.

Considering the close phylogenetic relationship, 1M6D (Crystal structure of human cathepsin F), 2P86 (cathepsin L protease from *T. brucei rhodesiense*) and 3BCN (Crystal structure of a papain–like cysteine protease) were selected for use as templates for homology modeling with quality factors of 88.688. Immunolocalisation analysis located TsCF1 on the inner layer of the cuticle and stichosome of the parasite, therefore it probably plays a vital role in nutrition uptake. The purified recombinant TsCF1 was used to immunize rabbit and the immune serum could recognize a 46 kDa band in soluble protein of muscle larvae.

After renaturation the TsCF1 recombinat protein demonstrated substantial enzyme activity to Z–Phe–Arg–AMC substrate with the optimal pH 5.5 and this activity could be inhibited by cysteine protease inhibitor E–64. Further analysis revealed the kinetic parameters of the recombinant peptidase to be Km=0.5091 μM and Vmax= 6.12 RFU/s μM at pH 5.5, and the IC50 value of E64 was 135.50±16.90 nM. These results indicated that the TsCF1 could be used as drug target and vaccine candidate for *T. spiralis*.

Biology, Host–Pathogen–Interaction and Immunology

Anti–tumor effects produced by excretory–secretory products of *Trichinella spiralis*

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Anti–tumor effects of *Trichinella spiralis* have been reported in mice infected with this parasite. But the anti–tumor active ingredients produced by *T. spiralis*. To further explore the active anti–tumor ingredients of *T. spiralis*, excretory–secretory products (ESP) of 6–day–old adult (Ad6) and muscle larvae at 35 days post infection (dpi) were investigated in the study.

Mice tumor model were established by grafting ascitic hepatoma H22. The tumor–bearing mice was administrated the two kinds of ESP by intravenous and intraperitoneal injection. The tumor–bearing mice were sacrificed after 12 day. The tumors were collected and weighed. The result showed that the ESP of Ad6 significant inhibited tumor expansion ($P < 0.01$). The effect of Ad6–ESP on peritoneal macrophages activation was also tested by immunofluorescence and flow cytometry *in vitro*. The results revealed that most peritoneal macrophages polarized to alternatively activated macrophages (M2).

Biology, Host–Pathogen–Interaction and Immunology

Trichinella spiralis p43, a Deoxyribonuclease Ⅱ but no activity

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Deoxyribonuclease Ⅱ (DNase Ⅱ) is a well–known acidic endonuclease that catalyses the degradation of DNA into oligonucleotides. 125 DNase Ⅱ–like protein family genes have been predicted in the *Trichinella spiralis* genome, and p43 of *T. spiralis* was only identified as a member of them on sequence level.

In this study, p43 gene of *T. spiralis* was cloned and expressed in a prokaryotic expression system and purified by nickel affinity chromatography. The results showed that the recombinant protein did not show enzyme activity. In order to corroborate this result, the native p43 protein from ES product of muscle larvae (ML) was purified by ion exchange chromatography and gel filtration chromatography.

The enzyme activity of the native protein still was not detected. In order to confirm the result, 2D nuclease zymography coupled with NanoLC–ESI–MS/MS method were performed. Interestingly, the spot of p43 protein did not show any activity.

The study demonstrated that *T. spiralis* p43 did not have DNase Ⅱ activity and might be a new type of DNase Ⅱ family.

§These authors contributed equally to the work.

Biology, Host–Pathogen–Interaction and Immunology

The roles of macrophage treated by excretory–secretory products from muscle larvae of *Trichinella spiralis* on the differentiation of C2C12 myoblasts

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The excretory–secretory products (ESP) released by muscle larvae (ML) stage of *Trichinella spiralis* have been demonstrated that involved in nurse cell formation. However, the ESP modulate molecular mechanisms of nurse cell formation remain unclear.

Macrophages exert either beneficial or deleterious effects on tissue repair, depending on their activation/polarization state. They are crucial for skeletal muscle repair, notably by acting on myogenic precursor cells. However, these interactions during the *T. spiralis* infection have not been characterized.

In the present study, the ability of ML ESP–treated macrophages to influence the differentiation of murine myoblasts and the mechanisms were investigated *in vitro* using the co–culture technique. The results showed that the expression of MRFs (MyoD and Myogenin) and MHC were reduced as compared with control group, indicating that ML ESP–treated macrophages inhibited myoblasts differentiation.

Our study suggested that ML–ESP can indirectly influence myoblasts differentiation by regulating macrophages activity, which provide new opinion for elucidate complex mechanisms of cell–parasite and cell–cell interaction during *T. spiralis* infection.

Biology, Host–Pathogen–Interaction and Immunology

New insights into the mechanism of *Trichinella spiralis* infective larvae invade the intestinal epithelium

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After being released from the capsule, *Trichinella spiralis* infective larvae penetrate the intestinal epithelium cells (IEC), this invasive process was the first and vital step during *Trichinella* infection. However, the invasion mechanism is not clear. Previous studies showed that the infective larvae didn't possess oral appendices or spikes, proposing that the invasion was mediated by larval invasion proteases combined with IEC membrane proteins, and bile as a trigger for invasion.

The aim of this study was to further investigate the larval invasive mechanism. The infective larvae were activated by 5 % pig bile at 37°C for 2h, and twenty BALB/c mice (10 animals each) were intraperitoneally inoculated with 300 bile–activated or saline–treated larvae. To our surprise, bile didn't show any promotion for *Trichinella* infection. None of 10 mice inoculated with bile–activated larvae were infected, while 8 of 10 mice inoculated with saline–treated larvae were successfully infected ($P < 0.01$). At 15 min after injection, the larvae were found in mesentery, liver, spleen, abdominal muscle, diaphragm and lung by compression and/or immunostaining method, the larvae were observed in intestinal epithelium at 12 h post injection.

Another experiment showed that the larvae penetrated through agarose gel layer with 4.5cm thick, larval penetration rates at 0.5 %, 1.0 %, 1.5 % and 2.0 % gels were 98.1 %, 87.2 %, 79.3 %, 62.8 %, respectively. The penetration rate had a descending trend (χ^2=435.763, $P < 0.01$) and an obvious negative correlation with the increase of gel concentrations (r=–0.989, $P < 0.05$). It is seemed that the larvae pose strong mechanical force to invade almost all kinds of host tissues. Additionally, the death rate of the bile–activated larvae at different time after culture *in vitro* was higher than those of saline–treated larvae (t=–4.954, $P < 0.01$). A novel but abnormal route of *Trichinella* infection was confirmed by intraperitoneal injection in this study.

Although we still don't know the exact migrating route how the larvae get into the small intestine, the results indicated that the larvae penetrated intestines mucosa not only mediated by invasion proteases, but also mainly by mechanical penetration. The bile might be only a trigger for larval development, but not for invasion.

2.3 Detection

Keynote

Trichinella spiralis and *Trichinella patagoniensis*–how to improve their detection in endemic regions?

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In some Latin American countries, human and swine trichinellosis is a problem of difficult solution due to the cultural habits of meat consumption and the factors that condition the presence of parasitism in the rural and wild environment, among them, pigs fed with raw meat residues, food stored improperly in the farms, lack of dead animal removal, failure in the control of rodents, precarious facilities for swine breeding and deficient elimination of residues that constitute an attraction for the animal species that live in the wild and direct themselves to urban areas in search of food. On the other hand, the informal offer of raw pork in different presentations and regional preparations (salami, bostton but, ham, dry salami) is very frequent and consumed by the population, without previous veterinarian control.

In Argentina, when people get sick due to meat consumption of pigs slaughtered in handcrafted manner, it is observed a bigger number of human cases during the winter season. If the human outbreaks are induced by pork meat sold illegally, the same happens all year long. During the period 2010/2015 an increase in human cases was detected produced by wild boar consumption after hunting tournaments. The animals that perpetuate the parasitism in nature and that were confirmed as transmitting agents are mainly pigs, wild boars, pumas (*Trichinella patagoniensis*), peccaries, armadillos, opossums, cats, dogs and synanthropic animals such as rats (*Rattus norvegicus*) and mice (*Mus musculus*).

The detection methods of parasitism in food animals use the quantification of larvae in the muscle tissue through artificial digestion, in slaughterhouses or licensed laboratories. There are detected deficits in the suitable performance of the prevention and control systems such as the low availability of materials like pepsin and hydrochloric acid, inadequate preservation and processing of the samples and lack of staff training to make diagnosis. The pork consumption increased from 2.5 kg/year/person in 2008 to 14 kg/year/person in 2014. Parallel to this, in 2008 there were 282 cases of human trichinellosis registered, while in 2014 there were 1067 cases. Between 2013 and 2014 there were 83 swine foci detected, with a prevalence of 0.09–0.16 % and an infection level of 1–3 larvae per gram (lpg).

In the foci there is a sporadic detection of pigs that fluctuate between 10–300 lpg and always proceed from pigs bred under deficient sanitary conditions. The origin of the swine foci diagnosis during the period 2010–2014 was 45 % in municipal and private laboratories, 35 % slaughterhouses, 16 % human outbreaks and 4 % seized products. In Argentina, when pigs are tested positive in the slaughterhouse, it is established that all animals proceeding from a swine foci must go to controlled slaughter. This affects economically the producer due to the fact that he is also forced to kill the highest value breeding pigs. During the last few years, there have been made several interventions in swine foci with the objective of applying a

serological diagnosis system in pigs ante mortem, in order to sacrifice only the parasitized animals allowing the negative ones to continue their productive cycle.

In several provinces of the centre and south of Argentina, wild boar hunting is performed for meat consumption and sporting purposes. In hunting tournaments it is important to develop awareness actions in the population in order to make the previous diagnosis before consumption, the training of the hunters should be directed to the making and referral of appropriate samples according animal species as well as the knowledge of prevention of this zoonosis. It is necessary to increase the direct and indirect diagnosis in areas where epidemiological data is scarce to get to know the circulating *Trichinella* species and identify the animals involved in the life cycle in different regions of America.

The training since the earliest stages of the educational cycle is vital for the acknowledgement of the risks of consuming meat without veterinarian control and the impact parasitism generates when it is lodged in the muscle fibers of the organism. The critical points to work in Argentina orient to monitor the biosecurity in farms, especially the circulation of wild animals, to make traceability from the farm to the fork and quality control in the slaughterhouse diagnosis, to increase controls in the different stages in commercializing the products of swine and wild boar origin to foment the regional brands in different places in the country.

The negative impact of trichinellosis in the population retracts consumption making the regional markets weak. To accompany the increase of pig meat consumption observed in the last few years, the national, provincial and municipal sanitary authorities should increase the control measures in order to avoid the negative association between pig and wild boar meat with disease.

Detection

Detection of antibodies specific for *Trichinella spiralis* by competition: one assay for a variety of animal hosts

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Antibody detection is used in research to follow experimental infections and in diagnostic work to detect infection by a variety of *Trichinella* species in a diverse array of animal hosts. Traditional assays for antibody detection rely upon secondary antibody reagents that are specific to the host species. We sought to develop an assay that would detect *Trichinella*–specific antibodies in sera of any animal host. Because conventional assays often employ L1 excretory–secretory antigens that are costly to produce, a second important goal of assay development was to reduce the expense of antigen preparation.

All species of *Trichinella* synthesize highly immunogenic glycans that incorporate the rare, dideoxyhexose, tyvelose. We coated plastic wells with crude L1 homogenate that is rich in tyvelose–bearing glycans. Tyvelose–specific antibodies were detected by incubating antigen with test sera mixed with biotinylated–monoclonal antibody of moderate affinity for tyvelose. Bound biotin was detected with avidin–peroxidase. Reduced binding of the conjugate indicated that tyvelose–specific antibodies were present in test sera. We tested sera from confirmed human cases of trichinellosis, a dog confirmed to be infected, sera from pigs experimentally infected with different species of *Trichinella*, and *T. spiralis* infected rat serum as a positive control. In addition, we screened sera from 486 cats and dogs from a local animal shelter.

Data from the competition assay were highly correlated with results from traditional assays of antibody detection that employ excretory–secretory antigens. Sera showing 40 % inhibition or more yielded reproducible results on re–test. Advantages of the competition assay include the inexpensive source of antigen and a single reagent that can be used for detection of antibodies in sera from any host.

Detection

Development and performance evaluation of enzyme linked immunosorbent assay and lineblot for serological diagnosis of *Trichinella* in humans and animals

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Trichinella spiralis is the causative agent of trichinnellosis, humans as well as several animals can be infected. Eight *Trichinella* species and several strains have been described. The most widespread and most important species is *Trichinella spiralis*, which occurs worldwide, but prefers the temperate climate zones. The sources of human infection are raw and insufficiently cooked or frozen meat products from domestic pigs and wild boars, horses and less frequently from bears, dogs and other animal species. Dried and pickled meat containing trichinellae can also be infective. The severity and duration of clinical manifestations depend on the infective dose and the rate of reproduction of the trichinellae. Symptoms may range from very mild to severe and depend on the number of larvae ingested and on the host's immunity.

According to Commission Regulation (EC) No. 2075/2005 all pigs slaughtered for human consumption shall be tested for *Trichinella* infection. During meat inspection muscle tissue samples (diaphragm, masseter) are collected and examined for *Trichinella* larvae by artificial digestion and microscopy.

An exception exists for animals from holdings that have been officially recognized as free from *Trichinella* or from regions presenting a negligible *Trichinella* risk. In these cases, monitoring programs are carried out in order to verify that the animals are effectively free from *Trichinella*. Serological methods can be used as part of these monitoring programs.

The aim of this work was to develop a serological assay to detect IgG and IgM antibodies against *Trichinella* in serum, plasma and meat juice. Native antigen preparations were used to coat 96 well microtiterplates and to print lineblots. For the detection anti–human IgG antibodies, anti–pig IgG antibodies or a protein A/G conjugate, able to detect IgG and IgM simultaneously, are used. The assays were evaluated with defined samples and an external evaluation at the national reference center in Bucharest, Romania took place. Samples used for the evaluation originated from humans, pigs, wild boars, foxes, badger and marten.

For the human assay values for sensitivity and specificity of > 95 % could be achieved. For the veterinary assays values of around 98 % for sensitivity and specificity could be achieved. Cut off values have to be adjusted for each species.

With the newly developed *Trichinella* antibody detection assays we now have a tool to diagnose *Trichinella* infection in humans and animals as well as to perform screening and seroprevalence studies. With the help of ELISA and Lineblots the assays can be done in high throughput settings as well as in resource limited laboratories.

Detection

Early serodiagnosis of trichinellosis by ELISA using excretory–secretory antigens of *Trichinella spiralis* intestinal infective larvae

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The excretory–secretory (ES) antigens from *Trichinella spiralis* muscle larvae (ML) are the most commonly used diagnostic antigens for trichinellosis because the ES antigens are easily prepared by ML cultivation, but detection rate of anti–*Trichinella* IgG antibodies did not reach 100 % until at least 1–3 months after human infection, there is an obvious time lag (window period) between *Trichinella* infection and antibody positivity. Intestinal infective larvae (IIL) are the first invasion stage during *Trichinella* infection, and their ES antigens are firstly exposed to the immune system and induce the host to produce antibody response.

The aim of this study was to evaluate the early diagnostic values of IIL ES antigens for trichinellosis. The IIL and ML were collected from small intestines and muscles of infected mice at 6 hours post infection (hpi) and 42 days post infection (dpi), respectively, and their ES antigens were prepared by incubation for 18 h. Anti–*Trichinella* IgG antibodies in sera of infected mice and patients with trichinellosis were tested by ELISA with IIL ES, IIL crude antigens and ML ES antigens. For the detection of anti–*Trichinella* antibodies in sera of mice infected with *T. spiralis*, the sensitivity of ELISA with 3 kinds of antigens was 100 % (25/25), but the specificity was 100 % (94/94), 58.51 %(55/94) and 100 % (94/94) ($P < 0.05$), respectively.

In lightly infected mice (100 larvae/mouse), specific antibodies were firstly detected at 8, 12 and 12 dpi, respectively; the antibody positive rate reached 100 % at 14, 20 and 24 dpi, respectively. In the heavily infected mice (500 larvae/mouse), specific antibodies were firstly detected at 10, 10 and 10 dpi, respectively; the antibody positive rate reached 100 % at 10, 12 and 16 dpi, respectively. When the serum samples of patients with trichinellosis were assayed, the sensitivity of ELISA with IIL ES, IIL crude and ML ES antigens was 100 % (42/42), 100 % (42/42) and 88 % (37/42), respectively ($P < 0.05$), and the specificity was 96.22 % (153/159), 94.34 % (150/159) and 83.31 % (142/159), respectively ($P < 0.05$). When the patients' sera collected at 35 dpi were assayed, the sensitivity of ELISA with 3 kinds of antigens were 100 % (22/22);

However, the sensitivity was 100 % (20/20), 100 % (20/20) and 75 % (15/20) ($P < 0.05$), respectively while the patients' sera at 19 dpi were assayed. Our results indicated that the ES antigens of *T. spiralis* IIL could be considered as the early specific diagnostic antigens for trichinellosis.

Detection

Evaluation of sample storage conditions for the recovery of *Trichinella* larvae from pork and horsemeat by the artificial digestion method

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The artificial digestion method continues to be the only reliable tool for the detection of *Trichinella* infection in carcasses to ensure food safety. Although the critical control points of the assay have been defined and can be effectively controlled for reliable performance, information is lacking on several aspects of the suitability of samples for testing.

The digestion assay has only been extensively validated using fresh muscle samples. The present study was designed to assess the suitability of fresh and frozen samples under various storage conditions for the recovery of *Trichinella* larvae. Samples sizes of 3, 5, 10, 20 and 100 g obtained from pig (ham) or horse muscle naturally infected with *T. spiralis* and *T. murrelli*, respectively, were used in the study. Baseline values for the number of larvae per gram (LPG) were obtained prior to subjecting samples to specific storage Treatments.

There was no significant change in larval recovery over time when samples were stored in whirl–pack bags or vacuum pack–bags and stored at 4°C for at least 8 weeks. The average LPG was also similar for each sample size regardless of whether samples were fresh or had been previously frozen at –10°C or –20°C. Doubling the primary sedimentation time in the assay had no effect on the number of larvae recovered. This study provides data to help define the range (scope?) of acceptable sample storage conditions for pork or horsemeat tested using the artificial digestion method for the detection of *Trichinella* larvae.

Detection

Diagnostic panel for foodborne pathogens

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The changes in farming practices towards bio–production, globalization of food market, global climate change and also the way of food processing facilitate the risks of widespread disseminations of foodborne diseases, including the pathogens of protozoa and multicellular parasites such as *Toxoplasma gondii, Trichinella spiralis* or *Taenia saginata*. These facts consequently induce the need of improvement of the diagnostic tools for foodborne agents in the final food products such as meat.

In relation to this problematics, our work is focused on the development of reliable comprehensive molecular diagnostic methods useful for rapid control of meat products on the market. For this purpose, we adopted high sensitive multiplex oligonucleotide ligation–PCR assay – MOL–PCR, representing the novel diagnostic platform based on magnetic microspheres and visualization realized via MAGPIX instrument for qualitative and quantitative readout of signal. This modern approach enables the simultaneous direct screening of complex samples potentially containing the DNA originating from number of different parasitic organisms.

To date, the specific molecular probes allowing the detection and capturing of targeted DNA from two parasitic worms–*Trichinella spiralis* (partial sequence of 18S rRNA gene) and *Taenia saginata* (partial sequence of mitochondrial COX1 gene) were designed. The calibration of the system and optimization of method is in process.

Detection

Faster and cheaper species identification of *Trichinella* muscle larvae (?)

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In Europe, four species of *Trichinella* are known to exist: *Trichinella spiralis, Trichinella nativa, Trichinella britovi* and *Trichinella pseudospiralis*. Their life cycles are separate but virtually similar. Each species has its typical geographic distribution and host animal range. For epidemiological purposes, and to enable understanding of e.g. cross–immunity, species identification of muscle larvae is of upmost importance. Because morphological differences between the species are small or non–existent, identification is based on molecular biology. Multiplex PCR identifying all species on a single run is a widely used method for identification of single larvae. The major drawback of the multiplex PCR method is that it is labour–intensive and costly. This is why e.g. in epidemiological surveillance of wildlife, often just 3 or 5 muscle larvae are identified from a single animal host. A cheaper method would enable the species identification of a greater number of larvae, thus possibly revealing more mixed infections, which are often seen also with current methodology.

Matrix–assisted laser desorption/ionization (MALDI) time–of–flight mass spectrometer (TOF) is an ionization technique allowing mass spectrometry analysis of biomolecules such as proteins. MALDI–TOF spectra are increasingly used for the identification of microorganisms. Bacterial cultures are ionized and their mass spectra analysed by computer software which automatically compares the spectra with profiles acquired from identified bacterial cultures archived in a “library”. In addition to bacterium, also fungus and even mosquito libraries have been created and utilized for identification of species with minor morphological differences. Species identification by MALDI–TOF is to be faster and cheaper than by alternative methods.

In order to test if there are species–specific protein differences between *Trichinella* species identifiable in MALDI–TOF spectra, we killed four mice from the FINPAR *Trichinella* mouse colony in Helsinki, each mouse infected with a different species of *Trichinella*. The mice were skinned and eviscerated in Oulu. Carcasses were minced and digested in artificial digestion fluid according to a standard procedure. Muscle larvae were collected in water and sent to Uppsala, where individual larvae were placed on a MALDI target plate and directly overlaid with 1µl of MALDI matrix. MALDI–TOF analysis was done using a Bruker microflex LT with Flexcontrol software. MALDI–TOF spectra were created as a first introductory opus in our *Trichinella* library.

The initial results are promising; there are regions in the spectra appearing potentially useful in identification of the 4 species. We need to test larvae of different origin before we hopefully can eventually claim that the method fulfils the high expectations we have in it. Further results will be presented at ICT 14.

Detection

Rapid detection of the foodborne pathogen *Trichinella* spp. by MALDI–TOF mass spectrometry

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Human trichinellosis occurs through the consumption of raw or inadequately processed meat or meat products containing larvae of the parasitic nematodes of the genus *Trichinella*. Currently, nine species and three genotypes are recognized, of which *T. spiralis*, *T. britovi* and *T. pseudospiralis* have the highest public health relevance. To date, the differentiation of the larvae to the species and genotype level is based on molecular methods, which can be relatively time consuming and labor intensive. The matrix assisted laser desorption / ionization time of flight mass spectrometry (MALDI–TOF MS) is an easy and rapid method used in predominantly bacterial, and as of late, parasite species identification. The method is based on the recognition of specific proteins of the microorganisms. As both the protein composition of each organism and the MALDI–TOF MS spectrum are unique, the obtained signature can be compared with a database of known microorganisms and the sample identified with high confidence. Here, a MALDI–TOF MS method was established for the rapid identification and differentiation of all known *Trichinella* species and genotypes.

A *Trichinella* specific protein extraction protocol was developed. Then, a reference spectra library was created using 29 *Trichinella* strains of all known species and genotypes. The generated spectra were evaluated by score value and composite correlation index analysis. Further, master spectrum dendrogram cluster analysis was performed.

The evaluation of the spectra generated by MALDI–TOF MS revealed a classification which was comparable to the results obtained by molecular methods. Also, each *Trichinella* species utilized in this study was distinct and distinguishable with a high confidence level. Further, different conservation methods such as freezing and conservation in alcohol and the host species origin of the isolated larvae did not have a significant influence on the generated spectra.

Therefore, the described MALDI–TOF MS can successfully be implemented for species level identification and poses a robust and convenient alternative to morphological genus and molecular species determination in a routine setting.

Detection

Epitope mapping of a strongly antigenic cystatin–like protein from *Trichinella spiralis*

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The artificial digestion method is widely used for the detection of *Trichinella* infection but costly and labour–intensive. The serological methods cannot replace artificial digestion methods because of the 'blind window' in which anti–*Trichinella* antibodies cannot be detected until 3–4 weeks post infection.

In our further studies, a candidate cystatin–like protein termed Ts–CLP which could be detected as early as 15 days post infection was identified. In order to screen the epitopes of Ts–CLP which could be used for diagnosis and vaccine candidates, the protein sequence was analyzed by B–cell epitope prediction software.

15 epitopes were selected based on the predicted scores, then amplified and cloned into pGEX–4T–1 vector. The recombinant plasmids were transformed into in *E. coli* BL21 (DE3) cells and induced with IPTG. The recombinant polypeptides were purified with Gluthathione Sepharose 4B. Western blotting and ELISA were used to evaluate the potential diagnostic value of the recombined epitopes. 12 of 15 polypeptides present high antigenicity and could be used as candidate antigens for diagnosis and vaccine development.

Detection

Initial examination of 6 pig slaughterhouses and a sample of wild rodents have not detected *Trichinella* in different towns of Antioquia, Colombia

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Trichinella is a nematode which infects mammals, including humans, pigs and rodents. More than eight *Trichinella* species have been described, but the most common species to humans is *Trichinella spiralis*. People become infected by eating undercooked pork meat with infective larvae. To date, this parasite has not been reported in Colombia, but the techniques used for diagnosis were not recommended by the International Commission on trichinellosis (ICT), so it is not accurate to say that it is a *Trichinella* free zone. Therefore, to verify if this pathogen is truly absent from Colombian territory, inspections are being made by the techniques that are authorized by the ICT in the laboratory of Veterinary Parasitology, Faculty of Agrarian Sciences at the University of Antioquia.

Evaluation of antibodies against *Trichinella* was performed by ELISA in 212 swine sera of different ages from 7 slaughterhouses in the department of Antioquia in 2014. Samples were analyzed with *Trichinella* PIGTYPE kit and any positive pig was detected. Furthermore, for direct detection, the enzymatic digestion test was performed in muscle to extract and concentrate the larvae. A sample of 100 pigs distributed in 5 towns of Antioquia was taken. Segments of diaphragm muscle and tongue (10 g per sample) were processed in groups of 10 animals.

None of the samples tested were positive. In parallel to this study, 150 wild rodents (*Rattus norvegicus*) from the Aburrá Valley were studied in groups of 30 animals. The diaphragm, masseter and tongue muscles were extracted for digestion test and larvae were not isolated.

In case that larvae would be found in the digestion test, it will be confirmed by multiplex PCR, which can determine the *Trichinella* species present. Thanks to the work of the Laboratory of Veterinary Parasitology of the UdeA, Colombia currently has an authorized laboratory for detection of this parasite, which is crucial for continuous monitoring and studies to corroborate the free status of *Trichinella* in pigs and possible risk factors for wild hosts.

2.4 Epidemiology

Keynote

The dynamics of *Trichinella spiralis* epidemiology: From down on the farm to living wild?

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Recent data on trichinellosis global prevalence and outbreaks, morbidity assessments, and food sources will be reviewed, along with key knowledge gaps. This will introduce the issue of free–range pig production and the potential exposure to sylvatic *Trichinella* spp. Data from 2010 to present reveal a continuing a long–term downward trend in global outbreaks and human cases. Most outbreaks have occurred in Argentina and in several European countries; the EU accounted for 63.6 % of cases during the period, and five Member States accounted for 86.9 % of these cases. As a health burden, trichinellosis has low impact (DALY, 76/billion persons/year), however, this assessment is hampered by the lack of long–term data on the extent and nature of chronic trichinellosis and its correlation to severity of the acute infection, especially in children. A WHO/FAO multi–criteria comparison of *T. spiralis* with 23 other foodborne parasites also rated *T. spiralis* low as a foodborne health risk, but ranked it first in impact on trade.

A previous evaluation of global data from 1986 to 2009 indicated domestic pigs were the source of infection in the majority of trichinellosis cases (53 %, compared to 42 % from wild animals). This meat source profile, however, appears to be shifting. In the 2010–2013 interval the global majority of cases were derived from game meat (54 %) while domestic pigs were responsible for 46 %. This shift has occurred mostly in countries that have markedly reduced the incidence of pig infections. Pork still accounts for the majority of outbreaks, however, in certain regions: in the EU, 68 % of cases in 2013 were attributed to domestic pork, the majority from Eastern Europe. In Argentina nearly all outbreaks are due to domestic pork, in contrast to Canada and South Korea in which all cases were attributed to wild animals. The percent of cases acquired from game meat in the USA has increased from 43 % to 89 % since 1997. *Trichinella spiralis* continues to be responsible for most outbreaks, and was acquired mostly from either domestic pork or wild boar. Worldwide though, sylvatic species such as *T. britovi, T. nativa T. murrelli* and *T. pseudospiralis* are often implicated in outbreaks attributed to wild animal meat, especially that of wild boars and bears.

The reduction in global incidence of trichinellosis due to pork is linked to the expansion of controlled management of commercial pig production. However, the increase of free–range pig production, especially in Europe and North America, has brought concerns that greater outdoor exposure increases the risk of infection with *T. spiralis,* (also *T. britovii and T. pseudospiralis* in Europe) from wild animal reservoirs. However, this risk is not well understood, often not supported with direct evidence, and epidemiological studies from different countries are sometimes contradictory. For countries without mandatory inspection of these higher risk pigs, research based on HACCP principles is required to develop the practical measures needed by free–range operators to maximize farm security.

Questions that should be addressed include:

- What is the sustainability of *T. spiralis* in a sylvatic cycle in the absence of re–introductions from the synanthropic cycle? Research studies have not always reached similar conclusions on this important question. There is, however, a consensus that locations with relatively high pig prevalence will also have increased wild life prevalence. These situations offer an opportunity for long–term studies on the persistence of *T. spiralis* in wild animals when there is also a concomitant effort to control farm pig infection.
- If *T. spiralis* is confirmed to be able to sustain an independent sylvatic cycle, this research program could provide needed epizoological information on important reservoir and vector host species of *T. spiralis*. Because of the increasingly common detection of *T. spiralis* (and *T. britovi*) in wild boars, and the population increases of this host species (e.g., Europe, USA), assessment of the risk from wild boars should be a priority. The role of rodents (e.g., rats, micro–mammals) should also be a research focus.
- A greater understanding of the circumstances allowing the spillover of sylvatic *T. spiralis* into a free–range pig farm is needed. This will require trace back of outbreaks in pigs produced outdoors to identify the specific sources of *Trichinella* (e.g., waste food, game meat disposal, carrion, etc.) and mode and degree of pig exposure to wild life. High–risk farm management systems (facilities, husbandry practices, etc.) need to be characterized. Trace back for research should be especially opportune in countries that require testing of outdoor reared pigs.

Research outcomes should be the ability to assess the potential for *T. spiralis* and *T. britovi* spillover into free–range farms, and the farm management and facility requirements needed to prevent it.

Epidemiology

The epidemiology of *Trichinella pseudospiralis* an elusive nematode

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Trichinella pseudospiralis (*Tps*) is one of the three species whose larvae do not encapsulate after muscle cell differentiation, and the only one infecting both mammals and birds. More than 40 years after its description, the epidemiology of *Tps* remains, at least in part, still enigmatic.

The aim of this work was reconsider the current knowledge of *Tps* biology and epidemiology by collecting the information available in the literature and in the website of the International *Trichinella* Reference Center. After artificial digestion, *Tps* larvae can be easily distinguished from those of the encapsulated species by their biokinetic, which is anterior end coiling for *Tps* and serpentine for encapsulated species. *T. pseudospiralis* was isolated from 17 mammalian species (3 marsupials; 2 rodents; 3 canids; 4 felids; 2 mustelids; 1 procyonid; and swine) including humans, and 6 avian species (1 eagle; 1 vulture; 1 crow; 3 night bird of preys) of Asia, America, Australia, and Europe. In the European Union, *Tps* has been documented in 18 out of 28 countries. Over the last 10 years, *Tps* accounted for 1.8 %, *T. spiralis* 51.3 %, and *T. britovi* 46.8 %, of 1,677 isolates from wild boar of Europe. The increasing number of reports of *Tps* is related to the increased use of the more sensitive digestion tests.

The number of mammals tested for *Trichinella* by digestion has been much higher than that of birds, thus the role played by birds in the epidemiology of *Tps* is still to be established. The cosmopolitan distribution of *Tps* suggests a role of birds in the spread of this parasite, but the identification of genetically different populations in different continents suggests also genetic isolation of continental populations in the last 1–2 million years. *T. pseudospiralis* has been also reported in domestic pigs of Bosnia Herzegovina, Croatia, Kamchatka and Slovakia, and in a wild boar farm of Italy. Other animal species, such as rodents, could be suspected as reservoir of *Tsp*; alternatively, the natural biomass of this species should be considered lower than that of the other *Trichinella* species.

Trichinellosis caused by *Tps* has been rarely documented in humans, in agreement to the scarcity of reports in animals. One human infection, probably acquired in Tasmania, and three outbreaks involving a total of 92 people, in Kamchatka, Thailand and France were reported. Recently, *Tps* has been suspected as the etiological agent of a human trichinellosis outbreak in Italy.

Epidemiology

Hunting dogs as sentinel animals for monitoring *Trichinella* spp. infection in wildlife

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Hunting activities can favour the spread of *Trichinella* spp. among wildlife. Canids are important natural reservoir hosts and have been frequently found infected by *Trichinella* spp. due to their scavenger behaviour. A common way for hunting wild boar is by dogs, which drive out and push wild boar towards hunters. The aim of the present work was to evaluate if the serological detection of anti–*Trichinella* IgG in hunting dogs can be a tool to indirectly monitor *Trichinella* spp. infection in wildlife. To this end, an ELISA and a Western blotting (Wb) using excretory–secretory antigens for the detection of anti–*Trichinella* IgG in dog serum samples were developed and validated. Furthermore, serum samples of hunting dogs were tested by the validated assays.

To validate the assays, serum samples were collected from 480 laboratory dogs and 43 pet dogs negative for *Trichinella* by ELISA with excretory/secretory antigens; 15 naturally infected dogs; and 6 experimentally infected foxes. The ELISA sensitivity and specificity were 100 % and 97.03 %, respectively. All sera from *Trichinella* infected dogs/foxes reacted by Wb with a three–band pattern ranging from 48 to 72 kDa. To evaluate the test reliability on the field, serum samples were collected from hunting dogs from 19 hunting districts of the Lucca province (Tuscany region, Central Italy), where *T. britovi* circulates among wildlife, and more than 5,000 wild boar are hunted per year. Out of 384 serum samples, 189 (49.2 %) tested positive by ELISA and of these, 56 (29.6 %) tested positive by Wb, showing an overall prevalence of 14.6 % (56/384) in the hunting dog population of the investigated area. All the 56 positive dogs were wild boar hunting animals.

The serological prevalence in hunting dogs was significantly ($p < 0.001$) associated with the hunting district, i.e. the higher the prevalence, the higher the average altitude of the hunting district. This is in agreement with previous investigations in Italy, which had shown that the prevalence of *T. britovi* in wildlife is higher in mountain areas than in low land areas. No statistically significant difference was observed when positive dogs were stratified by age, sex, race, and length of hunting activity. The results suggest that the circulation of *Trichinella* spp. among wildlife can be monitored by testing sera from hunting dogs, which could act as sentinel animals of *Trichinella* spp. circulation in wildlife.

Epidemiology

Study on the prevalence of *Trichinella* spp. in raccoon dogs (*Nyctereutes procyonoides*) in Brandenburg, Germany

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Trichinellosis is caused by nematodes of the genus *Trichinella* and an important meatborne zoonosis. Due to its distinct scavenging behaviour, the raccoon dog is of particular importance regarding the epidemiology of *Trichinella* in the sylvatic cycle in Germany. During the last two decades, the raccoon dog population steadily increased in the North–Eastern part of the country and has reached an average hunting bag of approximately 6,500 animals per year in the Federal State of Brandenburg (area 29,654 km²).

In this study in Brandenburg, Germany, a total of 1527 raccoon dogs were examined for *Trichinella* spp. between 2002 and 2014. All animals came from the national rabies control program. A 5 g mix from diaphragmatic pillars, masseter muscle and antebrachial musculature was examined for *Trichinella* larvae with the magnetic stirrer method according to OIE (2012). In positive animals, the parasite burden was calculated as larvae per g muscle weight and the *Trichinella* species was identified by means of a multiplex–PCR.

29 out of 1527 raccoon dogs (1.9 %) were found positive for *Trichinella* larvae. 26 isolates (90 %) were typed as *T. spiralis* and here, the parasite burden in musculature varied from 0.5–235 larvae per g (median 12.3). The other two isolates were identified as *T. pseudospiralis* (3.9 larvae per g) and *T. britovi* (210 larvae per g). A verification of *Trichinella* species was not possible in one case (2.7 larvae per g). *Trichinella*–positive raccoon dogs originated from 7 (Ostprignitz–Ruppin, Oberhavel, Barnim, Uckermark, Märkisch–Oderland, Oder–Spree, Dahme–Spreewald) out of the 14 investigated districts in Brandenburg.

The results of this study show that the 1.9 % *Trichinella* prevalence in the raccoon dog population in Brandenburg is significantly higher compared to the national average prevalence rate found during systematic meat inspection in wild boars (< 0.003 %) and a monitoring study in foxes in 2011 (< 0.3 %). The reservoir competence of the raccoon dog in the sylvatic cycle in Brandenburg is further emphasized by the high larval muscle burdens found for *T. spiralis* and *T. britovi*. Therefore, raccoon dogs and other wild animal species susceptible for *Trichinella* infection should be given special attention with respect to hunting practices (i.e. proper disposal of carcasses) to avoid the spread of this zoonotic parasite.

Epidemiology

Microsatellite analysis of *Trichinella britovi* isolates from the Mediterranean islands of Corsica and Sardinia suggests their different geographical origin

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In 2004 and 2005, two foci of *Trichinella britovi* appeared suddenly in domestic and wild animals of two restricted areas of the Mediterranean islands of Corsica (France) and Sardinia (Italy), considered until then to be *Trichinella* free. An epidemiological link between the two foci was suspected due to their geographic proximity and animal trade (pigs and dogs) between the two islands.

To investigate the origin of these foci, an average of 19 single larvae belonging to 11 isolates of *T. britovi* (3 from Corsica; 2 from Sardinia; 3 from continental France; 2 from continental Italy; and 1 from continental Spain) were genotyped at 6 loci containing microsatellites. Two polymorphic loci were used to investigate the genetic structure of the isolates. The two polymorphic loci showed the presence of 5 and 6 alleles with an average expected heterozygosity (He) of 0.25. Only one isolate from continental France showed both loci fixed to a single allele.

The test of the Hardy–Weinberg equilibrium showed that only 2 isolates (from continental France and Italy) displayed a significant departure from the null hypothesis ($P < 0.05$). The Fst index showed a consistent genetic differentiation among the isolates (average 0.206 ± 0.16 SD; range 0.782–0.005). This index displays the Sardinian isolates well separated from the continental and Corsican isolates. A similar pattern was obtained by the Bayesian analysis, which grouped the isolates in 8 clusters. The genetic relationships obtained by the UPGMA algorithm, showed the Sardinian isolates included in one cluster with an isolate from continental France; and the Corsican isolates in another cluster with two isolates from continental France and Spain.

In conclusion, these preliminary results show that the two Sardinian isolates are genetically different from both the continental (France, Italy and Spain) and Corsican isolates, suggesting an ancient introduction of *T. britovi* in this island, followed by the fixation of alleles scarcely represented in other areas of the Mediterranean basin. On the contrary, the detection in the Corsican isolates of alleles circulating in the continental Europe, suggests a recent introduction of *T. britovi* on this island. This conclusion is also supported by the detection of lower He values in the Corsican isolates (average 0.17) than in those from Sardinia (average 0.50), suggesting a drastic reduction of the genetic variability caused by the founder effect.

Epidemiology

Exposure of pigs to *Trichinella* spp. in three districts in Central and Eastern Uganda

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Since the 1980s, pork has become very popular in eastern Africa, with Uganda currently leading per capita consumption. Most of the pork is produced locally by smallholder pig farmers who ventured into piggery as a profitable income–generating activity. Yet, knowledge of good husbandry practices is limited and the majority of the pigs are kept in systems that allow free–ranging and scavenging. Nematodes of the *Trichinella* genus are known to enter the human food chain through the consumption of undercooked pork. In the East African Community, data on the presence of *Trichinella* spp. in domestic pigs is scarce and limited to erratic surveys using diagnostic methods with a low sensitivity such as trichinoscopy. This study aimed to determine if the domestic cycle of the parasite plays a role in Ugandan pigs and if consumers are at risk of contracting trichinellosis from eating undercooked pork.

In a cross–sectional survey conducted from April to July 2013 we sampled more than 1,000 smallholder pig farms in three districts in Central and Eastern Uganda. As part of a multi–pathogen assessment we collected pig sera, bio–data of the individual animals, herd composition and husbandry practices at production. The sera were examined using a commercially available enzyme–linked immunosorbant assay detecting anti–*Trichinella*–IgG. Positive samples underwent Western Blot for confirmation.

Seven percent (80/1124) of the sera tested positive and 97.5 % of these sero–positives originated from rural production systems. Only one third of these were confirmed IgG positive in the Western blot. Subsequently, 500 pork meat samples from four geographical clusters with a high seroprevalence were collected from November to December 2014 and examined using the artificial digestion method. All samples were negative for *Trichinella* larvae.

The presentation will discuss the implications of a sensitivity analysis, potential reasons for the high number of false–positives using the commercial ELISA as well as the suitability of indirect serological diagnostic tools developed in industrialized countries for utilization in extensive production systems in tropical countries.

Epidemiology

Microsatellite analysis of *Trichinella britovi* isolates from Latvia

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The prevalence of *Trichinella* spp. infection in wild carnivorous mammals of Latvia is more than 50 %, and *Trichinella britovi* is the prevalent species (>90 %) in both carnivores and omnivores. The aim of the present work was to investigate the genetic variability of *T. britovi* circulating in wild animals of Latvia by microsatellite analysis.

An average of 9 single larvae (range 7–12) from 52 *T. britovi* isolates were investigated at six loci. Two loci, TS10.10b and TS1380, showed a microsatellite polymorphism due to 5 and 7 alleles, respectively. A high degree of genetic variability was found for both polymorphic loci: i. 92 % of loci were polymorphic over all the isolates, whereas, only one isolate was fixed at both loci and only six isolates at one locus; and ii. the genetic variability evaluated by the expected heterozygosity was quite high (He = 0.423 for TS10.10B; and He = 0.388 for TS1380). Out of the 52 tested isolates, 86 % were in the Hardy–Weinberg equilibrium (HWE) ($p > 0.05$), and only one isolate was not in HWE for both alleles. The genetic differentiation among the *T. britovi* isolates was analysed by the Fst index, which shows an average value of 0.102 suggesting a reproductive isolation among individuals belonging to different isolates. This result is different from that found in *T. spiralis* isolates from Spain, in which a higher Fst index (0.254) was detected.

We can speculate that the transmission mechanism of these two species could be different and that the local parasite biomass and host species can play an important role. Based on published prevalence data, the *T. britovi* biomass appear to be extremely high in Latvia; whereas, the *T. spiralis* biomass seems to be 10–100 times lower in Spain. Furthermore, the most important reservoir hosts of *T. britovi* are carnivores, whereas, the most important reservoir hosts of *T. spiralis* are swine. In Latvia, the transmission pattern could be characterized by a high number of larvae, which reduces the loss of the genetic variability.

Epidemiology

Microsatellite analysis of *Trichinella spiralis* gene pool circulating in the Extremadura region of Spain

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In the Extremadura region of Spain, the prevalence of *Trichinella* spp. infection in hunted wild boar is about 0.18 % and approximately 77 % of the isolates belong to *Trichinella spiralis*. The aim of the present work was to investigate the genetic structure of *T. spiralis* isolates circulating in this restricted area (41,635 km^2, 8.25 % of Spain) by the microsatellite analysis of single larvae.

Thirteen loci were investigated on an average of 34 single larvae (range 9–36) from 35 isolates collected from 33 wild boars and 2 free–ranging pigs. Five loci (38 %) showed a reproducible PCR pattern due to microsatellite polymorphisms.

A low genetic variability was observed. Seven isolates showed a polymorphism at all the five loci; and 4 isolates were fixed at the same allele. The average number of alleles per polymorphic locus was 1.6 (range 1.0–2.2). A low expected heterozygosity was observed (average 0.18, range 0.00–2.20). The Hardy–Weinberg equilibrium showed a not significant departure ($p > 0.05$) in the 95 % of the loci. The genetic differentiation among the isolates showed a high Fst index (average 0.253, range 0.000–0.757), which suggests a low gene flow in the investigated area. This high genetic differentiation is unexpected for isolates supposed to belong to the same gene pool. The transmission of the infections due to few larvae (supposed by a larval burden of ≤ 10 larvae per gram in the diaphragm of 49 % of wild boar), and a rare occurrence of the transmission event in the wild boar population (supposed by the low prevalence, 0.18 % in wild boar, and 2.78 % in foxes), could be the two main factors influencing the observed genetic differentiation among the isolates.

The influence of the number of infecting larvae, which drives the pattern of genetic differentiation among the isolates at the transmission event, was also confirmed by laboratory experiments in mice in which the lower the number of infecting larvae, the higher the genetic differentiation of larvae in the mouse muscles.

Epidemiology

Wild carnivores, a major component of sylvatic reservoir for *Trichinella* spp. in Romania

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Wild carnivorous species represent an important component of the sylvatic reservoir of *Trichinella* spp. around the world. Romania is already recognized as an endemic territory of *Trichinella* infection in Europe and has large populations of these species among European countries.

In order to study the circulation of this opportunistic, sedulous and zoonotic parasite in Romania, 392 muscle samples were collected from 11 wild species belonging to the order Carnivora between 2011 and 2014. *Trichinella* sp. infection was revealed in 51 out of 362 examined foxes (*Vulpes vulpes*) (14.1 %, IC 95 %: 10.8–18.2), 3 out of 5 golden jackals (*Canis aureus*), (60 %, IC 95 %: 14.7–94.7), 1 of 2 wolves (*Canis lupus*), (50 %, IC 95 %: 1.3–98.7), 2 out of 11 wildcats (*Felis silvestris*), (18.2 %, IC: 2.3–51.8), one Eurasian lynx (*Lynx lynx*), and one beech marten (*Martes foina*).

All isolates collected from the jackal, wolf, wildcat, beech marten and lynx were identified by multiplex PCR (Pozio and La Rosa, 2003) as being *T. britovi,* while *T. spiralis* was found only in 37.2 % of the affected foxes.

Epidemiology

Trichinella in Corsica Island: when the parasite takes advantage of the slightest weak link

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Trichinella britovi emerged in the Taravo valley in South–Corsica in 2004 prior to which the Mediterranean island was considered as *Trichinella*–free. The parasite was detected during official inspection at the slaughterhouse in farms with traditional free ranging pigs for manufacturing delicatessen. Two pigs were identified at the slaughterhouse in March 2004, followed by 8 pigs in November.

Epidemiological studies conducted on foxes allowed the detection of a positive animal in the same valley among 74 foxes of the Island. Interestingly no wild boars were found positive during studies performed from 2006 to 2008 among 1881 animals controlled by direct tests. However, serological analysis carried on muscles fluid showed that 2 % of 1492 wild boars were seropositive (95 % CI: 1.4–2.9) showing an exposition to the parasite and thus the circulation at low level of *Trichinella* within Corsican wildlife. These results were strengthened by a serological study of farmers' dogs in Taravo (n=645) and several other valleys, revealing a seropositivity of 3 % (95 % CI: 1.9–4.8).

Due to reliable analysis performed by the accredited local routine veterinary laboratory, other positive out–door pigs were successfully detected at the slaughterhouse in 2010 (n=3), in 2011 (n=4), 2012 (n=6) and 2013 (n=2) in the Taravo valley and another valley of South–Corsica.

Moreover, local people are traditionally used to eat pork meat products well cooked, thus limiting the risk of human contamination in case of uncontrolled meat. Unfortunately, in April 2015 human trichinellosis was reported in continental South–France due to the consumption of raw Corsican figatelli bought via the internet.

So what happened? The sanitary investigation allowed the identification of the manufacturer, native to a village of Alta–Rocca, an area near the Taravo valley. This on–farm processor had built his business on the manufacturing of traditional saltings. Although he lives in an endemic area for pig trichinellosis identified as such since 11 years, he did not sent a part of his pigs to the slaughterhouse and so the pigs were not controlled, especially in February. This punctual lack of official control associated with bad information of the non–local customers caused a weakness in the control of the parasite, which took advantage of these circumstances.

Epidemiology

Prevalence and molecular characterization of *Trichinella* infection in wild animals in Israel

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Trichinella infection has been frequently diagnosed in wild animals in Israel over the past two decades. Annual examination of approximately 1000 wild boars reveals an overall incidence rate of between 1.9 % to 4.2 % with much higher incidence rates in the Golan Heights and Western Galilee (9 %–8 %). However, no infection of wild boars has been diagnosed in central and southern Israel. A survey conducted during 2005–2011, involving 809 golden jackals, resulted in extremely high infection rates of 36 %, 32 %, 28 % and 18 % in Jerusalem Mountains, Golan Heights, Carmel Mountains and Galilee, respectively. In the same survey, 523 foxes were examined, and the infection rates found to be 5 %, 20 %, 18 % and 6 % in these same areas were considerably lower than in golden jackals. *Trichinella* infection was also diagnosed in a few golden jackals in the central and southern parts of Israel. In order to determine the species of *Trichinella*, 42 isolates were examined by a duplex PCR that amplifies a part of the ribosomal internal transcribed sequence 1 (ITS1) and a part of the ribosomal expansion sequence V (ESV).

Out of 42 samples examined, 7 were from wild boars and 35 from wild canines (jackals, a wolf, and a fox). The PCR examination revealed two species, *T. britovi (Tb)* and *T. spiralis (Ts)*. In the wild boar isolates, two were *Tb*, and 5 were *Ts*. Within the carnivores, 30 isolates were *Tb*, two were *Ts*, and three were infected with both species. In order to examine the genetic variability of the isolates, a 550bp region of the ESV gene and a 518bp region of the ITS1 gene were analyzed. Comparison of the *Tb* and *Ts* ESV sequences revealed two short segments (12bp and 32bp) present only in the *Ts* sequences. Analysis of the ESV sequences showed that *Ts* isolates were 99.8 % identical, and *Tb* sequences were 100 % identical. Analysis of the ITS1 sequence showed one group of *Tb* (99.6 % identical), and two sub–groups of ITS1 within the *Ts* species (99.7 % and 96.9 % identical).

These results suggest that both species are genetically distinct and that there may be different subpopulations within the same species, in the same geographical region, as inferred from the genetic analysis.

2.5 Human Trichinellosis and Treatment

Keynote

Trichinophobia in Europe in the XIXth century

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To list human outbreaks which could have occurred in the past in France, the online catalogue of the French National Library was questioned with the following key–words: *trichine*, *trichinose*. Two peaks of publications in French were detected (1880–1889 & 1860–1869) and analysed. The 1880–1889 peak (6 monographs) was linked to the economical war between Europe and USA (see: "Dangerous meat? German–American quarrels over pork and beef, 1870–1900", U Spiekermann, Bull of the GHI, 2010, 46, 89–109).

Trichinella was certainly the scapegoat of a European fear facing a new economic power acting at the world level but, this period immediately followed the first human outbreak ever identified (1879) and reported in France (Laboulbène, 1881). In 1883, the authoritative monograph "*La trichine et la trichinose*" by J Chatin was published and the French authorities sent in Saxony, two famous physicians, Grancher (collaborator of Louis Pasteur) and Brouardel, to study the impressive Emersleben outbreak involving 52 deaths amongst 260 cases.

The description of the parasite cycle by Virchow (1858) and human disease by Zencker (1860) led to the publication of eight monographs in France during the 1860–1869 period; in particular "*Des trichines, à l'usage des médecins et des gens du monde*", a translation from German of Rudolf Virchow's "*Darstellung der Lehre von den Trichinen mit Rücksicht auf die dadurch gebotenen Vorsichtsmaßregeln für Laien und Aerzte*". Due to the numerous outbreaks, with a high lethality, reported from Germany, the parasite was quite familiar for the general population.

In the 1865 French slang dictionary, the verb "*trichiner*" meant "to consume delicatessen". The word "*trichine*" was used for "a little lady naturally involved in all social *cochonneries*, and which can poison the unwary consumer". But how such a concern by trichinellosis could have emerged though the parasite was not found in France?

Some political and economic events could have increased the angst of the disease. The liberal economical politic of the emperor Napoleon the 3rd led him to conclude, in 1862, a free trade agreement with Prussia. French farmers could have feared a massive importation of Prussian pork. The second point could have been a concern about German states and Prussia unification by the chancellor Otto von Bismarck. In cartoons of the time, *Trichinella* is clearly associated with Germany and German soldiers. In 1870, a war between France and Germany occurred and the Siege of Paris was particularly difficult for the population who had to feed on rats, cats & dog.

Human Trichinellosis and Treatment

Inference of the *Trichinella* species causing a human outbreak by serology

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In January, 2015, an outbreak of trichinellosis occurred in Genoa (Liguria region, Northern Italy). The epidemiological link among the patients was a dinner at a holiday farm near Genoa on December 31, 2014, where most of them had consumed 'beef' steak tartare. The symptomatology was mild and only 4 infected persons were hospitalized.

Signs and/or symptoms were observed in 73 % of the 52 (34 adults and 18 children) exposed individuals and 71 % had hypereosinophilia. Blood was collected from all 52 individuals about 30 and 60 days post infection. Serum samples were tested for anti–*Trichinella* IgG by three in–house tests: 1. an ELISA using excretory/secretory larva antigens (ESA); 2. an ELISA using crude worm extract antigens (CWE); and 3. a Western blot (Wb) using ESA.

As positive controls, serum samples from people infected by *T. spiralis* and *T. britovi* were used. Serum samples from 10 adults tested positive by the three tests and with high optical density (OD) values by CWE–ELISA and low OD values by ESA–ELISA; 1 serum sample from an adult tested positive by both ELISAs, but negative by Wb; serum samples from 25 persons (22 adults and 3 children) tested positive only by the CWE–ELISA with high OD values; and 16 persons (1 adult and 15 children) tested negative by all tests. Contrary to what has been found in other outbreaks, the OD values detected by ESA–ELISA decreased between 30 and 60 days post infection.

The epidemiological investigation did not allow to trace back the source of infection. However, the control of the invoice of the purchased beef did not fit with the amount of served 'beef', suggesting that meat of a different origin had been added to prepare the steak tartare. Given the high reactivity of 36 sera with CWE–ELISA, these sera were furtherly tested by Wb using CWE. Sera reacted strongly, but the Wb pattern was different from that of positive control sera. Serum samples from mice experimentally infected by *T. spiralis*, *T. britovi* and *T. pseudospiralis*, were tested by ESA–Wb and CWE–Wb. The CWE–Wb pattern of *T. pseudospiralis* infected mouse sera was very similar to that from persons of the Genoa outbreak, and different from those of *T. spiralis* and *T. britovi* infected mice. These results suggest *T. pseudospiralis* as the etiological agent of the outbreak. Wild boar infected by *T. pseudospiralis* has been already documented in the same Apennine area.

Human Trichinellosis and Treatment

The importance of objective clinical examination in patients with trichinellosis, registered in Braşov County, Romania, between 1983–2013

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Human trichinellosis is characterized by a polymorphism of subjective and objective clinical signs that are hampering the earliness of diagnosis and the initiation of antiparasitic therapy. It is important to know the peculiarities of objective manifestations in order to associate the antiparasitic therapy and the medication to support the systems and organs affected by this disease.

The study group has 1278 of human trichinellosis cases recorded during 1983–2013 in Braşov County, Romania. Objective clinical examinations show that cardiovascular manifestations are most frequent (12.32 %). Patients show these characteristics in this order: angina, palpitations, and shortness of breath. To these patients rhythm disorders were recorded independently or associated with previous manifestations: tachycardia, extrasystoles, atrial fibrillation, bradycardia.

Digestive disorders were found in 11.27 % of patients: nausea, vomiting, abdominal cramps with watery diarrhea, epigastric pain accompanied by heartburn, eructation, right upper quadrant pain spontaneously and on palpation, diarrhea. Compared to adults, children presented more frequently digestive symptoms. 9.06 % of the patients presented respiratory clinical signs: dry or wet cough, breathlessness, suffocation by the edema of the larynx. Bronchial crackles and crepitations accompanied congestive bronchopulmonary or pulmonary centers of infection. 2.42 % of the patients had obvious skin manifestations; some of them are located on the face or limbs, other being generalized. We mention kidney alterations in 1.68 % of the patients as follows: hyaline cylinders proteinuria, glomerulonephritis. Diabetes mellitus as a pre–existing condition was observed in 6 patients. The form of the disease was moderate in 4 cases and severe in 2. Hypothyroidism, observed in three adult patients, did not influence the progression of the moderate form of the disease. There were monitored 9 pregnant women during different periods of pregnancy. All developed moderate forms of the disease; their children were normally born and did not show any distress at birth.

Correlating the objective physical findings with the forms of disease that are recorded we observe that cardiovascular manifestations prevails in all moderate, moderate–severe, severe even in the mild form of the disease. These are followed by digestive and respiratory manifestations that are recorded at high parameters in moderate, moderate–severe and severe forms of the disease.

Human Trichinellosis and Treatment

The socio–professional configuration of trichinellosis patients for a period of 30 years

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Braşov County, located in Transylvania – mountain district in which one of the main occupations of rural inhabitants is farming, offers a wide field for the development of animal and human trichinellosis. Unlike counties in the south of the country, where people eat more vegetables, the population of Braşov county is consuming meat, mainly pork, but also beef, poultry, game, processed in different ways: roasted, boiled, fried smoked, brine, in some cases insufficiently or incorrectly cooked.

We aimed to analyze the socio–professional configuration of 1278 cases of trichinellosis from Braşov County – Romania, for a period of 30 years (1983–2013), in order to establish which groups of the population are at risk of illness. The study results will be used to find suitable accessible and diverse education methods of population to prevent this disease. Official statistics show that the incidence of trichinellosis values in Romania have a downward trend, primarily due to surveillance and control measures imposed by law in human and veterinary sector, but also due to the combined efforts of specialists in the field.

Analyzing the studied cases, the highest share is recorded in workers (35.93 %), housewives (12.01 %), pensioners (7.17 %) and children from their families (21.18 %). We affirm that of all children those aged between 0–1 years (0.21 %), 1–3 years (2.11 %), 3–6 years (2.95 %), 6–10 years (5.48 %), 10–14 years (7.06 %) were sick because of the neglect and ignorance of parents.

Interesting to point out is the percentage of patients among those working in public alimentation (2.95 %); although we believe that in their profession they were more circumspect about the possibilities of getting sick through consumption of infected pork. A smaller number of diseases was recorded among officials (1.90 %), engineers (1.48 %), technicians (1.26 %), farmers (1.26 %), teachers (1.05 %) and veterinarians (1.05 %). Physicians were ill with trichinellosis in a percentage of 0.42 %, by eating contaminated meat received in many occasions as "small gifts" (3 to 5 kg).

The military (0.74 %) and students (0.63 %) have a lower risk of disease because they eat in the mess hall or buttery, in organized system, where the origin of the meat is strictly controlled.

Human Trichinellosis and Treatment

Serological diagnosis of trichinellosis during two outbreaks in the territory of southeast Serbia

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Domestic pigs, raised and slaughtered in the backyards of small individual farms in Serbia still represent a major risk for human infection with *Trichinella spiralis.* In most cases small family outbreaks appear due to the consumption of uninspected raw or undercooked infected meat and meat products. The aim of this study is to present data regarding 25 patients, aged 2–75 years, who were involved in 2 out of 7 outbreaks that took place in Serbia in 2014.

Epidemiological investigations revealed that the diseased individuals consumed untested, undercooked pork (one outbreak that took place in District Bor) or raw pork sausages (another outbreak in District Zajecar). The rest of the food samples were *Trichinella* spp. positive by artificial digestion and larvae were identified as *T. spiralis*. Serodiagnosis was performed for 11 patients from the first outbreak (September 2014, the average age 50.54±12.24 years) and 14 patients from the second outbreak (November 2014, the average age 36.5±22.35 years) using the indirect immunofluorescent and ELISA immunoassays to detect the antibodies against *Trichinella spp*. The results showed that out of 25 patients, 22 were positive (88 %) and 3 (12 %) were negative for *Trichinella* antibody presence.

Out of the *Trichinella* positive sera, the sera of 6 patients demonstrated a concomitant positive reaction with *Toxocara canis* (*T. canis*) using the ELISA and Immunoblot Assays. In these patients, ocular fundus examination and chest x–ray were negative. With additional clinical examination, the existence of ocular and/or visceral *larva migrans* could be discarded. Seropositive patients for *Trichinella* met the case definition. The most prevalent symptoms included fever, asthenia and myalgia. In all cases standard Treatment with albendazole was administered, after which recuperation and cure of the diseased occurred. In order to have a second opinion and to validate the serology findings obtained from the Public Health Institute Nis, all serum samples were sent to the National Reference Laboratory for Trichinellosis, INEP where confirmatory testing was done using the WB method. For 6 sera in which antibodies against *T. canis* were found, it was established that they were positive on the presence of specific antibodies against *Trichinella*. Based on a proven high specificity of WB test (epitope in glycoproteins of 45, 49, 53 kDa from the excretory–secretory antigen of the muscle larvae of *T. spiralis*) serological diagnosis of trichinellosis was confirmed for all 6 patients.

Human Trichinellosis and Treatment

Trichinella infection in Serbia from 2011 to 2014

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Circulating among wild and domestic animals, *Trichinella* continues to be an animal husbandry problem and human health threat in Serbia. The rate of domestic swine infection with *Trichinella spiralis* gradually decreased from 0.026 % to 0.007 % between 2011 and 2014. This is a significant and constant decline comparing to the infection rate that characterized the first decade of this century (decrease from 0.14 % in 2001 to 0.02 % in 2010, average prevalence 0.07 %).

The prevalence calculated for 2014 corresponds to the prevalence that existed in the years before 1980 (less than 0,009), indicating that the lowest level of *Trichinella* infection, among domestic pigs in Serbia, is achieved nowadays. Infection presence among wild boars was monitored for the period 2012–2014 and revealed that infection with *Trichinella spp. (T. spiralis* and/or *T. britovi)* had the following rates: 2.32 % in 2012, 2.01 % in 2013 and 1.31 % in 2014. Despite the constant decline of the number of *Trichinella* positive domestic pigs, the number of human cases per year remains similar over time (in 2011, 2012, 2013 and 2014 there were 127, 45, 95 and 104 cases respectively), with no fatality. The incidence was 1.54, 1.74, 0.64, 1.31, and 1.39/100.000 inhabitants. During this 4 year period, there were in total 22 outbreaks, with 371 cases of trichinellosis.

The average number of cases per year was 93, which is a slight decrease compared to 138 cases in the previous 5 year period. Most of the outbreaks were small family epidemics. Sources of the infection were mainly uninspected pork and in one case horse meat, containing infective larvae. Annually repeating outbreaks of trichinellosis indicate insufficient awareness of the risk of the disease and suggest that further efforts should be made in terms of education and trichinellosis prevention.

Human Trichinellosis and Treatment

An outbreak of trichinellosis in Belgium associated with the consumption of imported wild boar meat

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Between November 17 and December 1 2014, nine patients were admitted to three different Belgian hospitals with peri–orbital swelling, fever, muscular pain, night sweats and high eosinophilia. Two weeks after the first patient was admitted, the diagnosis of trichinellosis was made on a muscle biopsy from one of the patients. All exposed persons were invited by the regional public health authorities to be clinically evaluated. In total, 16 patients were confirmed with clinical trichinellosis by serology and/or biopsy analysis. Multiplex PCR performed on three biopsies gave a *Trichinella spiralis* result.

All 16 patients had eaten dishes containing wild boar meat in three different restaurants during the 1st week of November. These restaurants had purchased wild boar meat from the same supplier on the same day and the suspected meat belonged to one batch of wild boar meat that had been legally imported from northeast Spain. After the diagnosis of trichinellosis was made, the Flemish Agency for Care and Health and the Federal Agency for the safety of the Food Chain (FASFC) were notified. Alert notifications were sent out and all relevant public health authorities were informed. Radio and TV broadcasts and newspaper reports alerted the public. Primary care physicians were asked to stay alert for signs and symptoms possibly related to trichinellosis and to ask patients about possible exposure. Paired serologic testing was also performed on three out of four asymptomatic persons accompanying confirmed cases and reporting the same consumption, but they stayed negative. After the press alert another seven patients were actively identified, but the possibility of other cases with less or no symptoms is not excluded.

FASFC conducted an investigation focusing on the supplier of wild boar meat. All wild boar meat from this involved supplier was recalled and examined for the presence of *Trichinella* larvae. Fifty eight samples of 21 different batch numbers (including samples from the suspected batch) of this Spanish wild boar meat were analysed by the magnetic stirrer digestion method, but all analyses were negative.

This is the first outbreak in Belgium since 1979. Although the source could not be confirmed, there is strong evidence that legally imported wild boar meat from a certified Spanish supplier caused the outbreak. The first signs and symptoms in the patients appeared after a median of 13 days (range: 6–22 days) after exposure; by the time diagnosis of trichinellosis was made, all suspected meat had been processed and consumed.

Human Trichinellosis and Treatment

Perceived barriers to ascertainment and reporting of human trichinellosis cases in the European Union/European Economic Area

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Human trichinellosis is a foodborne parasitic disease ranked in 2012 by FAO/WHO among the top 10 most important parasitic diseases for risk management. In the EU/EEA, surveillance of human trichinellosis is subject to under–reporting and under–ascertainment. An estimated 10–25 % of cases are missed; possibly more, considering the diagnostic challenges of chronic trichinellosis. In 2014, the European Centre for Disease Prevention and Control (ECDC) initiated a project to address perceived barriers to reporting and ascertainment of human trichinellosis in the Member States (MS).

Between 5 February and 30 March 2015, ECDC surveyed National Focal Points for food– and waterborne diseases in 31 EU/EEA MS. An expert panel of EU clinical microbiologists, epidemiologists and veterinarians developed a questionnaire to collect information on laboratory diagnosis, surveillance and reporting of human trichinellosis and to ask respondents to rank, according to the perceived importance in their countries, the 24 foodborne parasites defined by FAO/WHO as globally important. The data was analysed for the EU/EEA and by regions using Kendall's W test (coefficient of concordance) to assess agreement.

Of 31 MS, 22 (70 %) answered surveillance questions and 25 (80 %) answered the prioritisation ranking. Important factors affecting burden of human trichinellosis included effectiveness of national and EU prevention and control measures, the ability of the pathogen to cause local outbreaks, the cost of screening, cost of surveillance and economic impact to the individual and the community (Kendalls' W: 0.1421). Factors perceived as contributing to under–reporting at national level included inadequate reporting by clinicians and laboratories and the lack of feedback to data providers (Kendall's W: 0.0951).

Factors perceived as contributing to under–ascertainment included lack of clinicians´ awareness and inadequate diagnostic services (Kendall's W: 0.0800). Important barriers to reporting to EU level included lack of staff, lack of laboratory diagnostic capabilities and regional differences in data collection (Kendall's W: 0.0193). Only 4 of 21 countries reported laboratory participation in external quality assessment. Respondents ranked *Trichinella spiralis* and *Trichinella* spp. fourth and fifth in the prioritisation ranking (Kendall's W). Western European countries ranked *T. spiralis* second, after *Toxoplasma gondii*.

EU/EEA MS perceive human trichinellosis as important, but inadequate diagnosis and gaps in surveillance limit burden estimates and evaluation of prevention and control programmes. Actions to improve surveillance need to increase clinicians' awareness, support laboratory collaborations for correct diagnosis, and provide feedback to data providers.

Human Trichinellosis and Treatment

Epidemiology of trichinellosis in Lithuania 2002–2014

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Previous studies have shown marked increase of both domestic and sylvatic trichinellosis during 1990s and highly endemic state in the beginning of 2000s in Lithuania. The increase was caused by marked socio–economical changes in the country and it would be interesting to see what influence on *Trichinella* epidemiology had almost 15 years of stable socio–economic state.

During 2005–2014 annual incidence in humans remains similar as in the beginning of 2000s, accounting for 0.3–0.6/100000 population (389 human cases in total). Only 25 human cases occurred as sporadic, while the rest occurred in 34 outbreaks. However, there were few years when incidence was as high as 2.3–3.3/100000, indicating that trichinellosis still remains an important food–borne parasitic zoonosis in the country.

Both infected pork and meat of wild boars remains the main sources of *Trichinella* infection for humans, responsible for 47.6 % and 42.9 % respectively. For the remaining 9.5 % cases the source remains unknown. However in reporting period outbreaks of human infection occurred more often due to pork (58.8 %) than due to wild boar meat (26.5 %), indicating awareness of hunters for trichinellosis, and lack of attention of pig small–holders.

All known outbreaks occurred due to officially non–inspected meat. *Trichinella* prevalence in domestic pigs is decreasing considerably from 0.004 % to 0.0003 % during the last 5 years (approx. 0.7 million pigs are slaughtered and examined annually). As before trichinellosis in pigs occurs only in non–industrial pig farms and both *T. spiralis* and *T. britovi* are found. This is not surprising as biosecurity in these farms usually is low or very low, and there is a constant *Trchinella* pressure from sylvatic animals.

As before, prevalence in the main sylvatic hosts (red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*)) remains very high up to 40–50 %, while it has decreased slightly in wild boars to approx. 0.3 %. Despite high risk *Trichinella* was not found in any of 1000–2000 annually slaughtered horses in Lithuania. Molecular analysis of *Trichinella* species revealed transmission of the domestic *T. spiralis* to wildlife and transmission of sylvatic *T. britovi* to domestic pigs as it was happening 15 years ago.

This epidemiological situation in Lithuania indicates that trichinellosis remains highly endemic in sylvatic animals and due to lack of biosecurity and awareness of pig producers it poses a constant pressure for domestic pigs and also humans.

Human Trichinellosis and Treatment

Romania and trichinellosis: a never ending story

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One hundred forty years after the first outbreak registered in Lassy, Romania is still the most affected country in the EU by trichinellosis: 719 human cases out of a total of 1798 within the last five years (2009–2013). It is probably correct to assume that trichinellosis, was present in Romania, long before the discovery of *Trichinella spiralis* by James Paget in 1835, but its dispersion certainly differs upon the origins of the population that is concerned.

The analysis of early epidemiological data confirm the conclusions suggested by the available historical data: a strong socio–cultural determinant for the emergence of trichinellosis in Romania, through the particular food habits of German populations established a long time ago in Romania. As these populations became established mainly in specific areas of Romania (of Transylvania), this socio–cultural determinant also induced the specific geographic distribution of trichinellosis, with an elevated incidence rate in Transylvania; a distribution which was still observed in the 60's.

In contrast, the analysis of the last five years (2009–2013), shows no specific geographic distribution, with the human cases spread over the entire regions of Romania. Concerning the animal cases, in spite of a declining trend of *Trichinella* infection since the 1990's, the prevalence within the domestic pig population is still among the highest within the EU (0.16 % vs 0.0002 %).

2.6 Legislation and Control

Keynote

How to ensure a negligible risk of *Trichinella* in pig farming from a control perspective?

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A high level of biosecurity is the basic approach to ensure a negligible risk of several infections including *Trichinella*. This information should continuously be communicated to farmers through their organizations and veterinarians. Hereby, freedom from infection can be maintained for the benefit of the farmers, the pigs and the consumers.

Guidelines on what constitutes a high level of biosecurity are available see e.g. the International Commission on Trichinellosis' homepage. These guidelines are being updated from time to another to ensure that the most recent knowledge about effective advice is given in a language that can be easily understood by the farmers and their advisors.

In the EU, the concept of controlled housing is in use. This means a very high degree of biosecurity and requires a type of animal husbandry, where pigs after weaning are kept under conditions controlled by the farmer with regard to feeding and housing. There must be no contact with wildlife, and effective rodent control is a necessity. A set of procedures laid out in EU Regulation 216/2014 specifies in details the requirements set to a pig farm applying controlled housing.

This also means that a negligible risk status for a country or region is no longer recognized in an international context by the World Animal Health Organisation (OIE). Instead, such recognition is linked to compartments of one or more farms applying a high level of biosecurity. No testing for *Trichinella* is required for pigs raised in a controlled housing compartment.

The next step is to control the compliance with the biosecurity requirements. Here, a visit to the individual farm is essential. Such an inspection can be made by the authorities or a veterinarian as part of a veterinary visit.

In the EU, private standards are increasingly being used as means of controlling the biosecurity on–farm. This implies that a 3rd–party independent auditor pays a visit to the farm at regular intervals. During the visit, all requirements are being controlled and irregularities are noted and reacted upon.

In Denmark, the scheme is called Danish Product Standard. It was put in place because of a need to document to customers – including trade partners – that the legislation as well as the specific requirements from customers is complied with. The main focus is the key areas affecting animal welfare, meat safety and traceability in the primary production of pigs.

More than 95 % of Danish pig production is covered in this scheme. It can easily be updated with new requirements such as controlling the detailed requirements for high biosecurity.

Similar schemes are in place in many other EU Member States: QS in Germany, IKB in the Netherlands.

In some countries, there is no or limited tradition for auditing of biosecurity on pig farms making it more challenging to implement such a scheme – in particular if *Trichinella* is the only reason. Moreover, it will be more costly if herds and abattoirs are plenty and small compared to few and large. Here, it must be born in mind, that proper biosecurity is the most effective way to keep infections out. The recent epidemic of Porcine Epidemic Diarrhoea (PED) in the US is an example of how devastating infections can be in pig production. Hence, farmers should be informed about the value of having such biosecurity auditing systems in place.

Other approaches to ensure a negligible risk – which do not involve a visit to the farm – include surveillance for *Trichinella* by use of a reliable diagnostic method such as artificial digestion. The advantage of such an approach is the cost–effectiveness, because samples can be taken at the abattoir in relation to slaughter and processed at a low cost. A strict traceability system should be in place allowing trace–back to farms that have delivered test–positive pigs. It is being discussed internationally 1) whether serology can replace artificial digestion, 2) how large a sample of carcasses needs to be surveyed and found negative for a compartment to maintain its negligible risk status, and 3) what the reaction to an occasional finding of 1–2 positives should be – please see Codex Alimentarius Commission's homepage.

Auditing of biosecurity at regular intervals should be undertaken for a compartment to maintain its negligible risk status. The frequency should be risk–based and ideally include both announced and unannounced visits. In Denmark, controlled housing pig farms are audited minimum every 3 years. An audit costs around 290,00 € and is financed by levy funds paid by the farmers.

For pig farms not complying with controlled housing requirements, an auditing of biosecurity does not make sense – although auditing for animal welfare or organic production may be required. For such farms, testing of all pigs is required in the EU – and this is seen both as a way of controlling and early warning surveillance of a potential high–risk sub population.

Legislation and Control

Development of an alternative artificial digestion method for detecting *Trichinella* larvae in meat of domestic swine

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According to Regulation (EC) No. 2075/2005 (European Community, 2005), carcasses of domestic pigs, horses, wild boar, and other farmed and wild animal species susceptible to *Trichinella* infestation should be systematically sampled in slaughterhouses or game–handling establishments as part of the post–mortem examination. The magnetic stirrer method for pooled sample digestion is considered as the reference method of detection of *Trichinella* larvae for routine use. This reference method is based on pepsin, as digestive protease to release the *Trichinella* larvae from the muscle meat which are then identified under a microscope.

In recent years, compliance with the EU regulation has been hampered by frequent supply shortages of pepsin and variations in quality. Moreover, the use of pepsin powder and hydrochloride acid is a potential work safety issue. Therefore, an alternative artificial digestion method, the PrioCHECK® *Trichinella* AAD, has been developed and is now officially recognized as an equivalent method for artificial digestion by the Commission of the European Communities. With a standardized production of a recombinant proteinase, liquid components and a digestion performed at a slightly basic pH, the PrioCHECK® *Trichinella* AAD has clear advantages over the pepsin based digestion method.

The PrioCHECK® *Trichinella* AAD is comprised of three components, (1) Digestion Buffer, (2) Digestion Buffer Additive which provides an optimal digestion environment for the enzyme and the (3) Enzyme Solution. The digestion procedure is comparable to the reference method (magnetic stirrer method) and allows pooling of individual meat samples up to 100 gram followed by digestion at 60°C for 20 min under constant stirring.

The performance of the PrioCHECK® *Trichinella* AAD was evaluated in more than 70 digestion runs, including diaphragm samples from slaughterhouses, experimentally infected animals, and samples spiked with *Trichinella* larvae obtained from the European Reference Laboratory for Parasites (EURLP). The digestion process was demonstrated to be fully compliant with the guidelines requiring the digestion residue that remains on the sieve to be below 5 % of the starting meat tissue. All spiked samples were correctly classified as positive and the method was found to consistently identify 1–3 larvae in samples spiked with 3 *Trichinella* larvae. During the digestion process, the *Trichinella* larvae are inactivated but they are morphologically intact and can be used for further strain typing by PCR. The PrioCHECK® *Trichinella* AAD was subjected to the validation process of the EURLP and recommended as equivalent method for detection of *Trichinella* in meat of domestic swine.

Legislation and Control

Trichinellosis and pork production in Argentina: New aspects of control in an endemic region

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Trichinella infection is limited to some risks which include feeding raw, waste products or dead animals and exposure to infected rodents and wildlife. Swine production systems in total confinement can reduce or eliminate the risk of *Trichinella* spp infection in pigs, making the detection of *Trichinella* infection in animals raised under these conditions, unnecessary. Good swine confinement facilities, negative records of *Trichinella* infections at slaughter pig farm audits and monitor management practices are considered to greatly reduce the risk of infection.

In Argentina, there are breeding pigs in total confinement during all stages of production, with architectural barriers, biosecurity conditions and rodent control. The aim of this study was to investigate the serological and parasitological level of *Trichinella* spp in pigs raised in farms with Good Production Practices in an endemic country. Serum and muscles samples were obtained from slaughterhouses.

The serum was separated from the blood components and 1ml aliquots were stored at –20°C until processing. For parasitological analysis of muscle tissue, diaphragm samples (5 g) were collected at the time of slaughter. Samples were stored at 4 ° Cuntil laboratory analysis. 3075 samples of pigs were analyzed by ELISA test and artificial digestion. To determine the *Trichinella* spp infection status of animals, a *T. spiralis* ELISA suitable for detection of antibodies in sera samples of pigs was used (PrioCHECK *Trichinella* Ab).

Results obtained above or equal the cut–off of 15 PP (% positivity) were considered positive and were indicated as *Trichinella* spp. infected. 100 % of diaphragm muscles and 97.7 % of serum samples were negative. The serological prevalence was 0.06 %. Raising pigs in endemic trichinellosis areas such as Argentina, has made the pigs retain their negative image to the consumer. In a changing world, with a framework of increasingly rigorous international marketing, it is necessary to respond to market demands. The best management practices that minimize the risk of human infection from pigs must be documented and will be a future model control on farms, which will enable the differentiation of meat in retail outlets to protect consumer health. Approximately 30–40 % of pig production in Argentina is performed under conditions that do not include the risk factors associated with *Trichinella* infection.

Legislation and Control

Evaluation of the PrioCHECK digestion assay kit for the detection of *Trichinella* larvae in pork, horsemeat and wildlife

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The artificial digestion method using pepsin protease and hydrochloric acid is currently recommended for the detection of *Trichinella* larvae in muscle of infected animals. Recently, an alternative enzyme, serine protease, was employed in the development of a commercially available digestion kit (PrioCHECK Trichinella AAD). This assay uses a higher temperature (60°C) to kill the larvae during the digestion process, mitigating safety and environmental risks from the parasite. The present study was conducted to determine the performance of the PrioCHECK kit compared to that of the pepsin/HCl digestion.

Replicate samples of pork diaphragm and masseter, and of horse tongue and masseter were used with or without proficiency samples to compare the two methods for digestability and the recovery of larvae. Samples of 115 g of muscle were digested to evaluate the upper limit of the methods. Tissues of bear and other wild animals naturally infected with *Trichinella* were also used to compare the performance of the methods. The motility of the larvae was also assessed to compare the detectability of live and dead parasites.

The results of the study showed that the PrioCHECK kit when used according to the manufacturer's instructions was effective in detecting *Trichinella* infection in all samples that contained 0.05 or more larvae per gram. Although there was no significant difference between the kit method and the standard pepsin/HCl digestion method in the average number of larvae recovered from pork diaphragm, fewer larvae were recovered from samples of horse tongue by digestion using serine protease and additional clarification was usually required. The results of testing wildlife samples were similar for the two methods.

Legislation and Control

From parasite to patient: a quantitative risk analysis model for Trichinella

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Trichinellosis is caused by consumption of raw or inadequately cooked meat containing nematodes of the genus *Trichinella*. *Trichinella spp*. infection of domestic pigs and wild boar occurs through scavenging and/or feeding of infected offal. In many countries individual carcass control is mandatory, but high costs without positive findings in industrialised pig production asked for an alternative approach with the same food safety objectives. EU legislation (2006, adapted in 2014) allows risk based *Trichinella* testing of domestic pigs in the EU Member States. Additionally, risk based monitoring is being discussed for global trade of pig meat in the Codex Alimentarius. However, risk based *Trichinella* monitoring is still lacking a quantitative model to determine the risk of human infections. A quantitative microbial risk assessment (QMRA) could be helpful to assay the role and impact of measures that restrict the presence of *Trichinella spp*. in pork.

Recently, a basic model was developed to estimate the residual *Trichinella* infection risk from controlled housing pigs. This model used prevalence estimates in the primary production and the slaughter phase. However, it lacks distribution of *Trichinella* larvae within the carcass or between portions of pork, which may overestimate the actual infection risk. Moreover, a dose response for *Trichinella* to quantify human infections was not included in that model.

It was our aim to determine the human infection risks of *Trichinella* after consuming pig meat from different husbandry systems and from consumption of wild boar meat. We defined four basic questions: 1) how many false negative carcasses escape meat control at the slaughterhouse labs; 2) what does *Trichinella* testing on diaphragm mean with respect to other muscle types and how could we translate positive carcasses to the number of positive portions of pork; 3) how are *Trichinella* larvae distributed over pork portions and 4) how effective is cooking to inactivate *Trichinella* larvae in meat?

For our QMRA model, we collected experimental and survey data concerning *Trichinella* prevalence in wildlife, test sensitivity of carcass control, quantitative partitioning of edible pork parts, quantitative *Trichinella* distribution in host muscle types and heat inactivation by cooking. Using the collected data, we built a QMRA that includes *Trichinella* prevalence in wild boar and follows the whole chain of events leading to consumer infection risk. The developed QMRA allows evaluation of the risk of *Trichinella* infection from hunted wild boar and from two different types of pig holding (free roaming and controlled housing).The abstract must fit on a single A4 page (297x210 mm).

Legislation and Control

EN ISO 18743: Detection of *Trichinella* larvae in meat by artificial digestion method: the first international standard on parasites in food microbiology

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The artificial digestion/magnetic stirrer method is considered to be the “gold standard” for the detection of *Trichinella* larvae in muscle samples from pigs and other susceptible animal species intended for human consumption, because it has proven to give the most reliable results in validation studies. Though normative documents, e.g. the European Commission Regulation No 2075/2005, the World Organization for Animal Health (OIE) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2012, and the International Commission on Trichinellosis (ICT) Recommendations and Guidelines, 2012, describing such method, are available, an international standard to fulfill all requirements for accreditation is still lacking. In 2011, standardization of the artificial digestion method for the detection of *Trichinella* larvae in muscle samples was launched by the International Organization for Standardization (ISO). A panel of international subject–matter experts developed a first draft, which was shared with ISO and European Committee for Standardization (CEN) members. The final revised draft was sent for voting on April, 2015, and, when a consensus is reached, it will become an ISO/CEN Standard, namely *EN ISO 18743.*

Detection of *Trichinella* larvae in meat by artificial digestion method: This Standard will be “applicable to the examination of meat from domestic and sylvatic animal species, which can be infected by nematodes of the genus *Trichinella*”, and it must be used in conjunction with OIE and ICT guidelines. To ensure the robustness and performance of the assay, special attention has been paid in describing how to address the critical points in the procedure. Moreover, to help inexperienced users, the Standard includes two normative annexes (Annex A, on predilection muscles for selected animal species, which are recommended for digestion testing for *Trichinella*; and Annex B, on how to perform the assay on frozen samples) and two informative annexes (Annex C, a scheme of the method and figures of microscopic findings after artificial digestion; and Annex D, an example of laboratory worksheet for data recording after testing). The Standard will allow national authorities to harmonize official controls to international rules, in order to guarantee meat safety and to facilitate import/export trade. For these reasons it has been proposed to be considered as a “high profile standard”, a future “best seller” as a food industry–impacting document. Finally, EN ISO 18743 will be the first international standard on parasites in food microbiology, a very important step in this still neglected field.

Legislation and Control

Introduction of the Latex–agglutination test in Switzerland for the detection of *Trichinella* infections in pigs

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Since 2007, *Trichinella*–control of all slaughtered pigs as well as some other susceptible animal species is mandatory in Switzerland, except where meat consumption is intended for private use only. Laboratories performing the *Trichinella* testing have to follow the technical guidelines released by the Federal Food Safety and Veterinary Office (FSVO) in Switzerland.

These guidelines are in accordance with the EU Regulation 2075/2005. However, national validation is required prior to adopting any new method or apparatus to be used for the artificial digestion method in Switzerland. In the context of validating new diagnostic techniques and of a National Reference Laboratory activity, the Institute of Parasitology in Bern initiated the validation of the Trichin–L kit, an antigen detection kit based on latex agglutination, for pigs. In total 30 pig samples were spiked with 1, 5, 10, 100 and 1000 larvae of three different *Trichinella* strains *(T. spiralis, T. pseudospiralis, T. britovi)* and matched with 12 negative samples.

Additionally, two sets of ring trial samples from the Federal Institute for Risk Assessment (BfR) in Berlin with unknown status were analyzed. Of the 30 positive samples prepared in our laboratory, all 6 samples with 1 larva reacted negative. All the negative samples reacted negative in the test system. All positive (3, 6, 10 and 18 larvae per sample) and negative samples from the BfR were correctly identified with the Trichin–L test. The detection limit for all samples was limited to 3 larvae in our laboratory.

The solubilized antigens maintained their reactivity at 4°C for 6d. The Swiss authorities followed our recommendation to accept the Trichin–L kit for the detection of *Trichinella* larvae in pig samples. From all 23 laboratories performing *Trichinella* testing in Switzerland, only one is actually relying on the Trichin–L Test. The performance of this laboratory in the proficiency testing's was excellent. Other laboratories are interested in the method, but as they also analyze horses and wild boars with the artificial digestion method, the Trichin–L testkit should be validated with meat of horse and boar origin such as to extend the application range of the Trichin–L test for other consumption species.

Legislation and Control

Epidemiological situation of *Trichinella* and control measures in Switzerland

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Trichinella spp. has not been detected anymore in Swiss pigs or wild boar for many decades. The latest large epidemiological survey (2007/2008) on *Trichinella* spp. in pigs and wildlife showed that *T. britovi* is prevalent in Swiss carnivores with 1.6 % in red fox (*Vulpes vulpes;* total n° assessed: 1,298) and 27 % in lynx (*Lynx lynx;* total n° assessed: 55). In wild boar, no larvae could be detected, but antibodies were present in 0.2 % of the assessed animals (n=1,458). In none of the nearly 20,000 assessed domestic pigs of different housing and production conditions, *Trichinella* larvae or antibodies could be detected. As large carnivores act as indicator animals in Switzerland, a surveillance program is continued in those species to monitor prevalence of infection and the *Trichinella* species present.

Since 2007, all slaughter pigs, as well as all horses and all game meat intended not only for private use, have to be tested for infection with *Trichinella* spp. according to the EU Regulation 2075/2005. As National Reference Laboratory, the Institute of Parasitology Bern organizes the yearly mandatory proficiency testing (PT) for all laboratories performing *Trichinella* testing. The number of laboratories participating in this PT rose from 11 in 2001 to 23 in 2015. Over the years, an increase in the accuracy and sensitivity of *Trichinella*–detection could be observed. However, new laboratories usually require one or two PTs to obtain sufficient results. The change in 2011 in the PT scheme, from a merely qualitative assessment to a quantitative assessment, also led to a transiently higher number of laboratories that had to repeat the PT.

In conclusion, *Trichinella britovi* is present in Swiss wildlife, but domestic animals seem to be free of *Trichinella* infection. Appropriate surveillance systems and continuous training of the laboratories performing *Trichinella* testing help to continue documenting this epidemiological status.

Legislation and Control

Ranking of *Trichinella* species and other foodborne parasites

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International guidelines exist for the control of two foodborne parasites: *Trichinella spiralis* and the larval stage of *Taenia saginata*, also known as *Cysticercus bovis*. Lengthy incubation periods between infection and symptoms of most foodborne parasites challenge identification of the food vehicle that resulted in exposure. Only recently, however, foodborne parasites are being recognized and beginning to take on the global attention that they deserve in terms of their impact on public health.

The Codex Alimentarius working group on Food Hygiene (CCHF) asked the FAO and WHO in 2010 to review the current status of knowledge on parasites in food, their public health and trade impact in order to assess the need for a general guideline to control foodborne parasites in food products. In September 2012, FAO and WHO convened an Expert Meeting on Foodborne Parasites at FAO Headquarters in Rome to respond to this request from CCFH. An electronic questionnaire, prior to the meeting, resulted in a list of 95 potential foodborne parasites for consideration.

During the meeting, a final list of 24 parasites was selected for ranking their impact on mainly public health defined criteria, with specific vehicles of transmission identified for each parasite. These criteria were then weighted by the expert panel, according to perceived importance and a combined score based on published data and weight was computed for each parasite. Parasite scores were then ranked based on their scores. *Taenia solium* was ranked one, *Trichinella spiralis* seven, *Trichinella* species other than *T. spiralis* was ranked sixteen, and *Taenia saginata* nineteen.

Global ranking based on multicriteria analyses, as described here, do not necessarily account for regional variations. Therefore, in Europe, it is aimed to repeat this methodology as part of an European project approved in 2015 financed by COST called a European network for foodborne parasites (Euro–FBP). Ranking methodologies using multicriteria analyses as described here are being increasingly used to prioritise surveillance, control and research activities.

Legislation and Control

Retrospective analysis of proficiency testing results of Polish laboratories performing *Trichinella* examination of pig meat

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Each year in Poland approximately 18 million pigs are slaughtered, more than 40,000 horses, over 100,000 wild boars and few hundreds of nutrias. To ensure the safety of meat and meat products the carcasses of all those animals undergo veterinary examination. The examination for the presence of *Trichinella* is part of *post mortem* examination and is mandatory in Poland. According to EU legislation, laboratories performing official examination are obliged to implement the management system and accreditation. Both the accreditation process and Commission Regulation 2075/2005 requires laboratories to confirm competence in proficiency tests (PT).

Since 2005 PT were launched in Poland for laboratories performing digestion assay (over 800 of laboratories localized by abattoirs and meat plants). In total, in the years 2005–2014 for the PT over 4300 sets of samples were sent to laboratories. Each set consist of four samples: three positives and one negative. For the first run naturally infected wild boar meat was used. Qualitative assessment showed that 84 % of the laboratories were able to correctly identify contaminated samples. In 2006 only 25 laboratories were involved in PTs, positive results were obtained by 86 % of laboratories. In 2007 for the first time samples of pork meat spiked with known number of *Trichinella* larvae was used for PT.

The following levels of contamination were used: 10–15 larvae, 20–25, 30–35 larvae and blank samples. Overall in 2007 – 564 laboratories participated in PTs. Positive results were obtained by 455 laboratories, representing 80.7 % of them. Inconsistent results were reported by 109 laboratories (19.3 %). In 2008 samples were spiked with 8, 15 and 25 larvae. In the study participated 790 laboratories, satisfying results were obtained by 675 laboratories (85.4 %), 115 laboratories (14.6 %) reports incompatible results. In 2009, once again the number of larvae in the samples was reduced, to 5, 8–10, and 15–20 larvae.

Positive results were obtained by 753 of 822 labs, 69 labs reported unacceptable (8.4 %) results. In subsequent years 2010 and 2011, the proportions were 95.1 % and 95.0 % and the level of contamination of the samples ranged from 5 to a maximum of 17 larvae. Since the middle of 2012 the number of larvae was lowered to 1, 3 and 5 in positive samples. In 2012, PTs programme was modified, before changes 153 laboratories attend PT, 149 (97.3 %) of them reported satisfying results (in two regions samples were provided twice due to modified PT plan).

Overall, according to new plan PT samples were provided for 128 laboratories in 2012. Satisfactory results were obtained by 116 of them (90,6 %). In 2013–2014 over 607 laboratories participate in PT, negative results were obtained by 67 of them (23 %). From year to year decline in the number of laboratories presenting unsatisfactory results is observed even if at the same time the number of spiked larvae in the samples decrease significantly. These results clearly demonstrate progress in the quality of the meat examination.

Accreditation, PTs and corrective actions lead to systematic improve of food safety in Poland. Obtained results confirmed effectiveness of PTs as a tool to improve the quality of examination in field laboratories.

Legislation and Control

Is mainland France free from *Trichinella* infection of domestic pigs?

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In 1997, France implemented the official testing of slaughtered pigs for *Trichinella* larvae detection. A risk–based surveillance was set up in which all high–risk pigs (out–door reared pigs, sows and boars) and around 0.1 % of low–risk pigs (indoor finishing pigs) are tested. In mainland France (excluding the Corsica islands), more than 23 million pigs are slaughtered every year; most of them are indoor pigs. From 2006, half of the indoor pigs slaughtered have been exported to Russia. A modified testing scheme has been applied for these animals. The aim of our study was to estimate the sensitivity of *Trichinella* surveillance system in pigs in mainland France using a stochastic scenario tree model.

The probability of freedom for pigs under existing EU legislation (design prevalence of 1 case per million) was then considered using historical data. Results on the assessment of French diagnostic laboratories under quality assurance were integrated in the tree scenario. Three conservative scenarios were considered to take into account the difference of risk for trichinellosis in the high risk and low risk pig populations. Number of years since the last case detected in pigs or humans were used to estimate the annual probability of introduction. The only case detected in pigs in mainland France since the implementation of the surveillance system occurred in 2007.

For the different risk scenarios our model showed that the mean surveillance system sensitivities were equal or higher than 95 % in 2013. However, a confidence of freedom of 95 % was not reached in the same year. Nevertheless, that value might be attained within the next few years provided no pigs are found positive for *Trichinella* and the maintenance of a high sensitivity of surveillance system. Indeed, using a simulated dataset a confidence of freedom greater than 95 % was obtained from 2014 for all the risk scenarios.

3 Abstracts for Student Research Award

3 Student Research Award

Trichinella spiralis infection and transplacental passage in human pregnancy. Differences between low versus high parasite burden areas?

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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 were or might have been involved.

Parasitological and immunoserological (IS) parameters, clinical background and transplacental passage of *Trichinella spiralis* in six cases from areas with low (LPBA, n=2, PB with a range of 0.024–1.1ML/g) and high (HPBA, n=4, PB with a range of 2–23 ML/g) swine meat parasite burden (PB), were studied and compared. Patients and their children were followed through time, taking sera samples during pregnancy (when *T. spiralis* infection was confirmed), at delivery, newborn and/or infant (≥ 1 year–old). Detection of total immunoglobulins (IgGAM) and isotypes against antigens from muscle (ML) and newborn larvae (NBL) of *T. spiralis* were carried out in all serum samples by IS techniques.

Histological analysis of two placentas and umbilical cords did not reveal any abnormalities. The results are: 1– All pregnant women studied showed low symptomatology and absence of secondary manifestations, compared with other patients in the same outbreaks; 2– All patients gave birth to healthy newborns through full–term pregnancy; 3– The helminthocytotoxic activity against NBL occurred due to Abs present in LPBA pregnant women and progesterone dependent in the case of HPBA pregnant women; 4– Presence and titres of specific IgGAM and isotypes showed to be different in both areas; 5– Detection of specific IgE and IgA in sera from newborns of both areas, and IgM in those with LPBA revealed the occurrence of transplacental passage of this Igs and/or their production by the foetus; 6– Presence of specific immunoglobulins in sera from three out of four infants suggests that the transplacental passage of the NBL is possible; 7– Anti–ML–ESP *T. spiralis* continues to be present in the serum from Infant 4, even after eight years. IgG4 isotype was detected as well in his mother.

This result strongly supports the hypothesis that vertical transmission in humans is possible, thus infecting the foetus. Although a greater number of patients should be analyzed, the results presented herein contribute to better understand the complex host–parasite relationship in *Trichinella spiralis* infection during pregnancy.

Student Research Award

Automated digital imaging and analysis system for detection of *Trichinella* larvae in meat

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Trichinellosis is a meat–borne zoonosis with a worldwide distribution and an important public health hazard. In the European Union Regulation (EC) No 854/2004 stipulates that all animals intended for human consumption that are susceptible to *Trichinella* spp. have to be examined postmortem. Analytical details are specified in Regulation (EC) No 2075/2005, naming the magnetic stirrer method for pooled sample digestion the reference method. This method is based on the artificial digestion of meat samples, concentration of *Trichinella* larvae (L1) in the digest and visualization examination using optical utilities (i.e. trichinoscope or stereomicroscope).

A recent review and study by Riehn et al. (2013) identified the final step – visualization by humans – as the most uncontrollable and thus most error–prone step in the whole protocol. In daily routine there is no control whether the personnel pay full attention throughout the whole process of visually examining the digest or not. Closely related to this problem is the lack of evidence based documentation and archiving of results in official *Trichinella* examination. The analytical approach to solve these problems could be the digitalization of the visualization.

In our studies we designed a new, tube–like larval counting basin and replaced the hitherto used visualization systems (stereomicroscope or trichinoscope) by inverse microscopy as described by Makrutzki et al. (2014). The inversion of the optical path in combination with the new counting basin permits depiction of the whole examination area in one single image using an ordinary digital camera system. This facilitated – for the very first time – not only full documentation of the examination results but was also the starting point for the development of an automated digital imaging and analysis system for official controls on *Trichinella* in meat. In corporation with an industry partner a prototype of such a system consisting mainly of a digital full frame reflex camera, a 180 mm macro objective, a deflection mirror combined with an individual imaging analysis software was developed. Evaluating this prototype, camera settings, external factors of influence such as illumination of L1 and impact of the meat matrix were investigated and settings were adjusted accordingly.

First results of our study indicate that this automated digital imaging and analysis system enables evidence based documentation and archiving of examination results. The presented system may be able to totally substitute the „human factor" and, thus, to eliminate this serious error–source of the visual examination step in official *Trichinella* examination.

4 Abstracts for poster presentations

4.1 Phylogeny, Genomics and Proteomics

Molecular characterisation of *Trichinella* larvae isolated from wild boars withcorrelation to geographical origin of host

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Wild boars (*Sus scrofa*) are the main reservoir of human trichinellosis in Poland. Therefore, the high density of wild boar population is considered as the threat for a public health. Up to 2012 only two species *Trichinella spiralis* and *Trichinella britovi* in wild boars were identified. However, species identification of larvae alone is not sufficient to confirm the suspected source of the outbreak. Therefore, recognition of differences between larvae of one species could be very useful for epidemiological investigators. The aim of a study was to determine intraspecies genetic differences in larvae isolated from wild boars in selected provinces of Poland.

For the study *Trichinella* isolates from 90 wild boars were used. DNA was isolated from five single larvae from each animal. In PCR, 5SrDNA intergenic region and COX1 mitochondrial DNA were amplified. Positive amplicons was used for sequencing. In total, 240 sequences of 5SrDNA and 220 sequences of COX1 were obtained. Phylogenetic analyses of consensus sequences were performed by the Neighbor–Joining method with Jukes–Cantor model in Geneious7.0.6.

The aligments of obtained 5SrDNA and COX1 sequences with reference sequences of all *Trichinella* species from GenBank showed occurrence of two genotypes of *T. spiralis* and six genotypes of *T. britovi* in 5SrDNA, and also two genotypes of *T. spiralis* and fifteen genotypes of *T. britovi* in COX1. The *T. spiralis* genotypes Ts1 and TsA were detected at the majority of larvae isolated from wild boars gained in all polish provinces. The genotypes Ts2 and TsB were detected only in North–West provinces. The *T. britovi* genotypes Tb1 and TbA were detected in all regions of the country, whereas, the less prevalent genotypes Tb2–Tb6 and TbB–TbR occurred only in few areas. The highest differentiation in *T.britovi* was found in North–East Poland. Detection of six genotypes of *T. britovi* in 5SrDNA analysis and fifteen genotypes in COX1 analysis showed higher differentiation inside the species compared to *T. spiralis,* where two genotypes in 5SrDNA and COX1 analysis were detected.

The identified genetic relationship between larvae of the same species could be helpful in epidemiological investigation, however other genes should be additionally examined.

Phylogeny, Genomics and Proteomics

Comparative Proteomics analysis of three developmental stages of *Trichinella spiralis*

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Trichinella spiralis, an intracellular parasite nematode, can cause severe foodborne zoonosis that is known as tichinellosis. The life cycle of *T. spiralis* consist of adult (Ad), muscle larvae (ML) and newborn larvae (NBL). However, studies are not available on the protein profiles in different developmental stages of the parasite. In the present study, proteins from lysates of Ad, ML and NBL were identified by iTRAQ.

The results showed that a total of 4701 proteins were identified and the number of up–regulated proteins in NBL was higher than that of other two groups. Then the protein profiles from Ad, ML and NBL were compared in pairs. Primary two clusters of the differently expressed proteins were determined with the functions regarding immune regulation and parasite invasion. Further investigation of the transcriptional levels of several genes from the differently protein profiles using quantitative PCR showed the identical results to the iTRAQ analysis. The function of these differently expressed proteins in regulation of development and host–parasite interaction should be further studied.

Phylogeny, Genomics and Proteomics

Fluorescent two–dimensional difference gel electrophoresis and mass spectrometry for the identification of species–specific *Trichinella spiralis and T. britovi* antigens

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Until 2012, in Poland, two *Trichinell*a species: *T. spiralis* and *T. britovi* were known to be prevalent among domestic and wildlife animals. In the last years two additional species *T. pseudospiralis* and *T. nativa* have been identified in wild animals in Poland. *T. spiralis* is the etiological agent of most human infections, however, outbreaks of human trichinellosis caused by other species: *T. britovi, T. nativa*, *T. pseudospiralis* have been reported world–wide. In the past years in Poland, the epizootic and epidemiological status of trichinellosis has changed due to the significant increase of the wild boar population and consumption of wild boar meat instead of pork. Therefore human trichinellosis has remained an unsolved problem of public health. Wild boar meat remains the primary source of *T. britovi* infection for humans. In recent years an increase of human trichinellosis caused by *T. britovi* was reported in several European countries. Up to now, it has not been possible to differentiate *Trichinella* species serologically, these methods are not appropriate for early and species–specific diagnostics. Nevertheless, diagnostics could be greatly improved if the infecting species could be identified serologically. Therefore, information about antigens, that are common or unique to different *Trichinella* species, is required to aid the development of species–specific diagnostics.

In the present study we applied fluorescent two–dimensional difference gel electrophoresis (2–D DIGE) technique, mass spectrometry and 2–DE immunoblotting to uncover common and unique excretory–secretory (E–S) proteins produced by *T. spiralis* and *T. britovi* muscle larvae that hold promise for species–specific diagnostics. A total of 22 proteins including potentially immunogenic and proteins produced only by one of the two *Trichinella* species were subjected to mass spectrometry for protein identification.

In the *T. spiralis* E–S proteome, the specifically appearing protein spots were identified as a glycoprotein p43 and serine proteases. The species–specific protein spots appearing only in the *T. britovi* E–S proteome were found to contain a 5'nucleotidase, serine protease and 49 kDa E–S antigen, which all were identified in multiple protein spots. The shared antigens were identified as gp43 and different protease variants. Our results also demonstrate the value of 2–D DIGE as a versatile tool to compare secretomes of different *Trichinella* species for pinpointing factors contributing to the interaction with the host.

Phylogeny, Genomics and Proteomics

Identification of *Trichinella spiralis* early antigens from excretory–secretory products of adult and newborn larvae by two–dimensional gel electrophoresis and immunoblotting

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The excretory–secretory (ES) proteins of *Trichinella spiralis* play an important role at host–parasite interactions and have been suggested as vaccine candidates for the control and prevention of trichinellosis. Although the ES proteins of muscle larvae (ML) have mainly been used in serodiagnosis of trichinellosis, the serologic tests still lack sensitivity in the detection of the early stage of infection.

In this study, a global Proteomics approach was used to analyse the ES proteins from *T. spiralis* adult and newborn larvae. Following two–dimensional gel electrophoresis and immunoblotting of ES proteins, liquid chromatography–tandem mass spectrometry (LC–MS/MS) was used to identify the protein spots.

The results showed that the representative gel spanning at a pH 4–7 range of ES products produced by the 3–day–old adult worms (AD3) and 6–day–old adult worms/newborn larvae (AD6 + NBL) consisted of approximate 500 distinct polypeptide spots. More than 70 of these proteins including DNAse Ⅱ, Cystatin and serine–type proteases were found to be immunogenic by western blot analysis using a serum from experimentally infected swine at 26 and 60 days post infection.

The identification of these antigens provides potential candidates for the early diagnosis of trichinellosis and for the development of a vaccine against this parasite.

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Phylogeny, Genomics and Proteomics

Adaptive properties and activity of proteolitic enzymes of the Arctic isolates of *Trichinella* under experimental inoculation of laboratory rodents

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An effect of the adaptation level toward non–specific host of the first stage juveniles of *Trichinella nativa* onto the activity of proteolitic enzymes (aminopeptidases, trypsine, chymotrypsine, subtilizine, prolil endopeptidase, cathepsin–like cysteine proteases *et cet*.) was studied. Biomass of juveniles was obtained through the inoculation of white mice, rats and rabbits with *Trichinella* invasive stages obtained from the muscles of polar bear, wolf and wolverines hunted at the Arctic territories of Far Eastern Federal District of Russia.

Proteolitic activity was estimated in the somatic extracts and secretory–excretory products of the *Trichinella* juveniles obtained from muscular tissue of laboratory hosts. To test the enzymatic activity short synthetic peptides were used with C–terminal chromogenic p–nitroanilid substrate of original design or commercial preparations (Bachem®, Switzerland) including: Z–Glu–pNA, Ac–Phe–pNA, Bz–Arg–pNA, Z–Phe–Arg–pNA, Z–Ala–Phe–Arg–pNA, Glp–Phe–Ala–pNA), Z–Ala–Ala–Leu–pNA, Glp–Ala–Ala–Leu–pNA, Z–Ala–Ala–Pro–pNA, Z–Gly–Gly–Pro–pNA. Substrate cleavage with the delivery of p–nitroanylid resulted in changed absorbance, what was detected in spectrophotometer at 410 nm.

The similarity in the spectra of proteolitic enzymes between studied Acrtic *Trichinella* isolates (all belonging to T2 = *Trichinella nativa*) was reported. In the same time significant difference in ability to hydrolyze substrates of chymotrypsine and other proteases was reported. Such differences in activity were especially pronounced in the *Trichinella* strains not adapted to the non–specific hosts. In such cases the activity of proteolitic enzymes increases many–fold and provokes pronounced changes in the structure of host muscular tissue, delay in capsule formation (up to complete absence of encapsulated juveniles and their consecutive dying–out).

High level of proteolitic activity correlates with low postchallenge antibody level against *T. nativa* antigens detected with enzyme immunoassay of inoculated rodents, what was considered as an indication on immunosuppressive action of proteases on the humoral antibody responsiveness. It seems that significant differences in the pathogenicity between *Trichinella* isolates are determined by the level of proteolitic activity of enzymes emitted by the juveniles localized in muscular tissue.

Phylogeny, Genomics and Proteomics

Modified primers 37F and 42R for amplification of cytochromoxydase I mitochondrial gene of *Trichinella*

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In attempt to find nucleotide differences between the *Trichinella* populations of the same species, a partial sequence of mitochondrial gene of cytochromoxydase I was amplified and sequenced. Primers 37F and 42R proposed by Hu et al. (2002) for ascaridid and rhabditid nematodes were used as a basis for primer design and the oligonucleotide sequence was changed to correspond to the trichinellid Cox1 mtDNA: the primers 37F_Tri (GCAGTAAATTTAGAATTTAAAC) and 42R_Tri (CCTAATATTCATGGTGTTCATA) were proposed. DNA template extracted with Promega® Wizard columns from *Trichinella* juveniles was obtained by digestion with artificial stomach juice of massive samples of host tissue and was used for amplification with these new primers.

Approximately 1,300 bp amplicon was obtained for 8 *Trichinella* strains. The Cox1 mtDNA sequences of two Russian strains of *Trichinella spiralis* (those from Chukchi sledge dog and wolf from Sakha–Jakutia) were found to be identical, but differed in 2 bp from Cox1 mtDNA sequences of *T. spiralis* from Central Europe and USA (our data for the strain from Czech Republic and deposited sequence for complete mitochondiral genome of *T. spiralis* – AF293969).

Interestingly, one of these substitutions is nonsynonymous, changing amino acid composition. The nucleotide differences were found between two groups of Russian strains of *Trichinella nativa*: strains from Chukchi Peninsula (hosts: polar fox, bearded seal and stray cat) differ in 4–5 nucleotides from three strains: *T. nativa* isolated from the brown bear hunted near Kolpashevo township (Tomsk region, Western Siberia) and *T. nativa* samples isolated from stray cat and red fox hunted near the city of Voronezh (Central part of European Russia). In the phylogenetic trees obtained with different methods of analysis (maximum parsimony, neighbour joining) these two groups of *T. nativa* formed stable clades with bootstrap support over 80 %.

We can presume that newly designed primers are effective for amplification of Cox1 mtDNA and provide some data for the understanding of differences between strains of *T. spiralis* and *T. nativa*.

Phylogeny, Genomics and Proteomics

Screening and identification of early diagnostic antigens from *Trichinella spiralis* intestinal infective larvae by immunoProteomics

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Trichinella spiralis muscle larvae are released from their capsules in the stomach, and activated into the first–stage intestine infective larvae (IIL_1) by exposure to intestinal contents or bile at 0.9 hour post infection (hpi). The IIL_1 larvae invade host's intestinal epithelium, and undergo the first molting to develop IIL_2 at 10 hpi, then molt three times to adults during 10–31 hpi.

The IIL_1 are the first invasion stage of *T. spiralis*, their excretory–secretory (ES) antigens are firstly exposed to the immune system and induce the host to produce specific antibody response. The aim of this study was to identify the early diagnostic antigens and vaccine target antigens from IIL_1 ES antigens. *T. spiralis* IIL_1 was collected at 6 hpi and their ES proteins were identified by SDS–PAGE and Western blot analysis.

The results showed that six protein bands (92, 52, 45, 35, 32, and 29 kDa) were recognized by sera from mice infected with *T. spiralis* at 10 days post infection. Then, the 6 bands were analyzed by using shotgun LC–MS/MS. Total 55 proteins of *T. spiralis* were identified, the molecular weight varied from 16.32 to 109.28 kDa, and the pI ranged from 4.36 to 9.64. Out of 55 proteins, 31 proteins were annotated according to Gene Ontology Annotation. Twenty–eight proteins (90.3 %) had hydrolase activity (e.g. peptidase activity, nuclease activity and serine hydrolase activity).

Several proteins, such as serine protease, putative trypsin, deoxyribonuclease II family protein, and antigen targeted by protective antibodies could be used as the early serodiagnostic candidate antigens for trichinellosis. Our study provides new insights for screening early diagnostic antigens from intestinal stage larvae of *T. spiralis.*

Phylogeny, Genomics and Proteomics

Immunoproteomic profile of *Trichinella spiralis* adult worm excretory-secretory antigens recognized by early infection sera

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Trichinella spiralis muscle larvae develop to adult worms 31 hours after infection, live in the intestinal mucosa and persist for days or weeks in different hosts. At intestinal stage of *Trichinella* infection, adult excretory–secretory (ES) antigens are exposed to the immune system and elicit the host to produce the specific anti–*Trichinella* antibodies. Hence, *T. spiralis* adult ES antigens have the potential for early serodiagnosis of trichinellosis. The purpose of this study was to identify the early serodiagnostic biomarkers and vaccine target molecules from *T. spiralis* adult worm ES antigens.

T. spiralis adult worms were collected at 48 hours post infection and their ES antigens were analyzed by SDS–PAGE and Western–blotting analysis, then the immunoreactive band was subjected to shotgun LC–MS/MS analysis in combination with bioinformatics.

Results showed that only one protein band (33 kDa) was recognized by sera from mice infected with *T. spiralis* at 8 days post infection. The shotgun LC–MS/MS analysis identified 23 proteins and clustered into 10 unique proteins, with molecular weights varying from 28.13 to 71.62 kDa, and pI values ranging from pH 5.05 to 9.20. Some enzymes (e.g. serine protease, adult–specific DNase II, peptidase, multi cystatin–like domain protein) were found to be highly expressed. According to Gene Ontology analysis 6 proteins were annotated, of which 5 proteins (83.33 %) had hydrolase activity.

The results provide a valuable basis to find early diagnostic antigens and vaccine candidates for trichinellosis.

4.2 Biology

Cloning and bioinformatics analysis of Thioredoxin peroxidase gene TsTPx1–3 from *Trichinella spiralis*

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The intracellular parasitic nematode, *Trichinella spiralis,* is known as the pathogen of the severe foodborne zoonosis, trichinellosis. During the parasite's rapid growth and generative propagation phases, high oxidative stress exists in the environment of their cells. Thioredoxin peroxidases are a ubiquitous family of anti–oxidants that could reduce H_2O_2 to water and hydroperoxides to alcohols. However, thioredoxin peroxidases in *T. spiralis* have not been identified by now. Here, the open reading frame (ORF) cDNA sequence of TsTPx1–3 was cloned by RT–PCR from *T. spiralis* muscle larvae and were then analyzed by bioinformatics.

The results showed that the lengths of ORFs of TsTPx1–3 were 747, 588 and 594, which could encode proteins with the theoretical molecular weight of 28.2 ku, 22.1 ku and 22.4 ku. TsTPx1–3 protein sequences showed about 60 % identity with thioredoxin peroxidase from other parasitic nematodes. All the deduced proteins predicted no signal peptide sequence, while they all contained a AhpC–TSA domain and a 1–cysPrx_C domain. Quantitative PCR results showed that TsTPx1–3 was expressed in adult (Ad), muscle larvae (ML) and newborn larvae (NBL) stages of *T. spiralis*. TsTPx1 was higher in ML, TsTPx2 and TsTPx3 were higher in Ad3. The TsTPx1–3 gene fragments were then cloned into a pET30a vector and recombinant pET30a–TsTPx1–3 were transformed into BL21(DE3) to induce expression.

Results showed that recombinant TsTPx1–3 proteins were highly expressed with 33.8 ku, 27.7 ku, 28 ku, respectively. The recombinant TsTPx1 protein existed as an insoluble body, while recombinant TsTPx2–3 proteins were existed in soluble form. Western–blot indicated that recombinant TsTPx1 protein can be recognized by *T. spiralis* infected swine serum whereas recombinant TsTPx2–3 proteins cannot be recognized by the same serum. Enzyme catalytic experiments showed that recombinant TsTPx1–3 proteins could deoxidate H_2O_2 and the ability rose with increasing protein concentration and growing time. This research will lay a foundation for further study of thioredoxin peroxidase of *T. spiralis*.

Biology

Cloning and identification of a putative aquaporin from *Trichinella spiralis* (TsAQP)

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Vaccination as one of prevention strategies against the *Trichinella spiralis* infection in hosts is an ongoing effort. However, no ideal vaccine candidates have been identified until now. Therefore, identification of more effective antigens in essential life stages of the parasite as vaccine candidates is of importance. In the present study, we identified a novel aquaporin gene in *T. spiralis* (TsAQP), and the potential antigenicity of TsAQP was evaluated by prediction of epitopes.

The results revealed that a total of 11 post–translational modification (PTM) sites falling into 4 categories, including *N*–glycosylation sites, casein kinase II phosphorylation sites, protein kinase C phosphorylation sites and *N*–myristoylation sites, were predicted in the protein. TsAQP belongs to membrane intrinsic protein with high hydrophobicity and the main hydrophobic domains were distributed at amino acid positions 21–43, 54–71, 83–91, 107–121, 163–174, 187–200 and 242–261, up to 38.5 % of the protein. The TsAQP protein mainly consisted of helixes (39.58 %) and loops (50 %). The advanced structure of TsAQP using homology modeling method showed that the protein was formed from 6 membrane–spanning domains connecting by 5 loops. Based on these analyses, 6 potential B–cell epitopes and 4 potential T–cell epitopes were further predicted. The mRNA expression levels of TsAQP gene in muscle larvae (ML), newborn larvae (NBL) and adults (Ad) were then analyzed by real–time quantitative PCR using GAPDH as reference gene.

Results showed that the TsAQP gene could express in all the three stages with a significantly higher expressed level in NBL compared that in Ad ($P < 0.05$). However, the expression of TsAQP gene in ML and Ad was not significantly different in statistically ($P > 0.05$). All the results suggested that TsAQP could be a promising candidate for vaccine antigen against *T. spiralis*.

Biology

Inactivation of encysted muscle larvae in pigs using Mebendazole

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We evaluated the effect of 4 anthelminthic Treatments on the viability of *Trichinella spiralis* encysted muscle larvae (ML; 60dpi) in infected pigs. Muscle larvae were isolated from pigs after Treatment with levamisole (8mg/kg, daily, 5 days), mebendazole (50mg/kg, daily, 5 days) , doramectin (0.3mg/kg, single IM injection), and moxidectin (0.5mg/kg, single pour on); larvae were inoculated into mice to assess viability. Only mebendazole Treatment of pigs rendered ML non–viable, as no ML were recovered from mice inoculated with larvae from the mebendazole treated pigs.

Since mature nurse cells containing older larvae may have reduced exposure to systemically absorbed anthelmintics than circulating larvae and larvae in younger nurse cells, analysis of the effect of mebendazole on larvae from shorter and longer term infections was conducted. Analysis revealed that mebendazole Treatment of pigs (50mg/kg, daily, 5 days) rendered all ML non–viable regardless of age of the nurse cell (45, 60, or 100 days post infection). Treatment regimens with mebendazole were then investigated in *Trichinella* infected pigs through analysis of effect of length of Treatment with a fixed dose of mebendazole on encysted ML.

Mebendazole Treatment of pigs with 250mg/kg over 3 days (83mg/kg/day) or 5 days (50mg/kg/day) reduced numbers of ML recovered from pigs, and rendered ML non–infective to mice, while mebendazole Treatment of pigs with 250mg/kg in a single dose was not effective in reducing ML numbers recovered from pigs or in impacting ML infectivity to mice. This procedure provides a means to evaluate the efficacy of various anthelmintic Treatments on the viability of *Trichinella* ML in pig tissues, and identified mebendazole as an anthelmintic which renders *Trichinella* ML non–infective.

Since risk from *Trichinella* significantly impacts acceptance of pork from pasture–raised pigs, these data provide a means, especially for producers of these high–risk pigs, to eliminate the potential of *Trichinella* transmission from infected pork.

Biology

Infectivity of *Trichinella spiralis* muscle larvae recovered from pig carcasses

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Trichinellosis is an endemic disease in Argentina, with pork being the most important animal species usually involved in human outbreaks. Pig farming and home slaughtering as well as home sausage making, are some very rooted traditions in Argentina, that when are not made properly, contributes to the perpetuation of this disease. The production of free–ranging pigs has increased in many countries, including Argentina. Pig farming with low sanitary and hygienic conditions, where animals are fed with food scraps or other forms of meat–containing animal wastes, allows *Trichinella spiralis* to infect domestic pigs.

The aim of the present work is to assess the role played by porcine infected meat in the perpetuation of this parasite in the environment. A male pig, hybrid (*Landrace* x *Yorkshire*) was infected per os with 2,000 muscle larvae of *T. spiralis*. In order to prepare the inoculums, larvae were recovered from a laboratory mouse by enzymatic digestion. The pig was allowed to acclimatize to the new environment, prior to parasite infection. At 120 days post inoculation (p.i) the pig was euthanized and eviscerated, according to bioethics rules. The carcass was cut into two identical pieces. One of the carcasses was placed on the surface of a plastic container which had previously been filled with soil. The second carcass was buried in another box. Both of them were covered with fine mesh wire and were located in an enclosed area with adequate ventilation, exposed to sunlight, and protected from rain. Weekly, muscle samples were taken from each carcass and were subjected to artificial digestion.

The recovered larvae were counted and inoculated to mice (300 larvae/mouse). After 6 weeks, mice were euthanized and digested to confirm the intensity of infection. The reproductive capacity index was calculated for each mouse. The study lasted 9 weeks, with a mean temperature of 16ºC and the humidity ranging from 88 % to 36 %. Infective capacity remained for 5 weeks in the samples belonging to the carcass that was on the surface, and 4 weeks in the samples belonging to the carcass that was buried. There were no significant differences between groups ($p>0.05$). Our study demonstrates that *T. spiralis* has the ability to remain infective in pig carcasses for several weeks under natural conditions, which would be a key link in the epidemiology of the disease.

Biology

DsRNA–mediated silencing of Nudix hydrolase in *Trichinella spiralis* inhibits the larval invasion and survival in mice

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RNA interference (RNAi) technique was widely used in variety of organisms, including parasites, as a powerful tool for gene functional study. The aim of this study was to investigate the functions of *T. spiralis* Nudix hydrolase (TsNd) during the larval invasion of intestinal epithelial cells (IECs), development and survival in host by RNAi. The TsNd–specific double–stranded RNA (dsRNA) was designed to silence the expression of TsNd in *T. spiralis* larvae. DsRNA were delivered to the larvae by soaking incubation or electroporation. Silencing effect of TsNd transcription and expression was determined by real–time PCR and Western blotting, respectively.

The infectivity of larvae treated with dsRNA was investigated by the in vitro larval invasion of IECs and experimental infection in mice. After being soaked with 40 ng/μl of dsRNA–TsNd, the transcription and expression level of TsNd gene was inhibited 65.8 % and 56.4 %, respectively ($P < 0.05$). After being electroporated with 40 ng/μl of dsRNA–TsNd, the transcription and expression level of TsNd gene was inhibited 74.2 % and 58.2 %, respectively ($P < 0.05$). Silencing TsNd expression by both soaking and electroporation inhibited significantly the larval invasion of IECs in a dose–dependent manner (r_1= –0.96798, r_2=–0.98707). The invasion rate of the larvae soaked with 20, 30, 40, 50 or 60 ng/μl dsRNA–TsNd for 18 h was 54.4 %, 44.7 %, 39.2 %, 35.3 %, and 32.7 %, respectively; while the invasion rate of the larvae treated with lip2000 or untreated was 61.7 % and 63.1 %, respectively.

The invasion rate of the larvae eletroporated with the above–mentioned dose of dsRNA–TsNd for 18 h was 54.4 %, 44.7 %, 39.2 %, 35.3 %, and 32.7 %, respectively; while the invasion rate of the larvae untreated was 58.3 %. Mice inoculated with larvae soaked with TsNd dsRNA displayed a 49.9 % reduction in intestinal adult worm burden and 39.9 % reduction in muscle larval burden compared with the mice inoculated with untreated larvae ($P < 0.05$). Mice infected with larvae electroporated with TsNd dsRNA displayed an 83.4 % reduction in intestinal adult worm burden and 69.5 % reduction in muscle larval burden compared with the mice inoculated with untreated larvae ($P < 0.05$), indicating that electroporation had a higher efficiency than soaking in inhibiting the larval development and survival in mice. Our results showed that silencing TsNd expression in *T. spiralis* inhibited significantly the larval invasion and survival in host.

4.3 Host–Pathogen-Interaction and Immunology

Developmental profile of immune cells in mice infected with *Trichinella spiralis* during intestinal phase

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Cellular immune response of the murine infected with 300 larvae of *Trichinella spiralis* during the intestinal phase was studied. Firstly, the recovery rate of intestinal worm was analyzed. The results showed that intestinal worms were completely eliminated at 17 day post–infection (dpi) and large numbers of newborn larvae were reproduced from 5 dpi to 9 dpi with a high peak at 7 dpi.

Then, development profile of immune cells in mice infected with *T. spiralis* was investigated from 1 dpi to 17 dpi. The number of $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes of the infected mice were increased during the whole intestinal phase, except CD4+ T lymphocytes on 7 dpi, during which the number of the cells were reduced. Interestingly, the ratio of $CD4^+/CD8^+$ was lower than the control groups indicating that *T. spiralis* can inhibit the host immune response.

In contrast with T lymphocytes response, during the intestinal phase the numbers of B lymphocytes were increased until 11 dpi. In addition, *T. spiralis* stimulated the proliferation of T and B lymphocytes during the intestinal phase, except $CD4^+$ T lymphocytes on the 7 dpi, during which the depression of proliferative response of T lymphocytes to Con A were observed. Additionally, the macrophage cell population significantly reduced at the early stages and they were recovered till the 11 dpi.

These data may benefit for us to better understand the development of intestinal immune response in mice infected with *T. spiralis*.

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Host–Pathogen-Interaction and Immunology

Immunomodulatory properties of various life stages of *Trichinella spiralis* and muscle larvae excretory–secretory products

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Helminths and their products can suppress the host immune response, benefiting parasite survival. *Trichinella spiralis* can establish chronic infections in a wide range of mammalian hosts, including humans and mice. The life cycle of *T. spiralis* is completed in a single host, and includes three main stages: adult worms (AD), newborn larvae (NBL) and muscle larvae (ML). During different phases of parasitic growth, *T. spiralis* interacts with the host immune system to evade or dampen the immune response. Recently, several studies have shown that infection with *T. spiralis* reduces the severity of various immunity–related diseases, including autoimmune Type 1 diabetes, experimental colitis, autoimmune encephalomyelitis, and airway allergic inflammation. Here, our aim was to study the effects of *T. spiralis* adult worm (AD), newborn larvae (NBL), muscle larvae (ML) and ML excretory–secretory (E–S) products on the human macrophage cell line THP–1 *in vitro*.

Macrophages are one of the initial components of an immune response and can have an influence on shifting the type and magnitude of the subsequent immune response. Monocytes were differentiated into macrophages and cultured with different parasite stages and E–S products, with or without LPS. We assessed the impact of the individual stages and E–S products on the profiles of cytokines secreted by the cells and analyzed the differences in kinase phosporylation profiles.

A reduction in expression of the cytokine IL–8 and the chemokines MCP–1, GROα, and SERPIN E1 was observed with cells cultured with AD, NBL and ML parasite stages using the Human Cytokine Array. We observed increases in s–ICAM, MIF and IP–10 levels after culture in the parasites presence. Regarding induced and repressed pathways, we noticed many differences pointing to contrary effects of the main life stages of parasite and excretory–secretory products; especially regarding the level of phosphorylation in ERK1/2, Akt S473, Akt T308, p53, p70, STAT3, and STAT6. Some data indicated greater immunosuppressive properties for excretory–secretory products compared to the whole parasite.

Host–Pathogen-Interaction and Immunology

Modification of the *Trichinella spiralis* intestinal settlement after antibiotic Treatment

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The intestinal microbiota is vital for shaping the local intestinal environment. Microbiota is also related with the immune response and metabolism of nutrients. Many parasites use the intestine as the final settlement site or as a stage for development in their life cycle. The interaction between microbiota and intestinal parasites is still little known. Both the microbiota and parasites have coevolved within the host establishing themselves environmental regulatory networks that allow them to live in balance. Modification of this balance could have a directly impact on the survival of the parasite. We have assessed the influence of the modification of microbiota with an aminoglycoside antibiotic (streptomycin) on the intestinal settlement of the parasite *T. spiralis*.

First of all we carried out an *ex vivo* discriminatory experiment in order to demonstrate that streptomycin is not an anthelmintic product, by directly treating the infective stage (L1 muscle larvae) of *T. spiralis* with different streptomycin doses (1, 2 and 5 mg/mL). None of them showed any effect on larval infectivity. In the *in vivo* assays our results demonstrated that changes of intestinal microbiota induced by previous antibiotic Treatment substantially modify the course of a subsequent infection by *Trichinella*.

Host–Pathogen-Interaction and Immunology

SiRNA–mediated silencing of Nudix hydrolase in *Trichinella spiralis* results in the reduction of larval infectivity

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Nudix hydrolase (Nd) is a widespread superfamily, which is found in all classes of organism, hydrolyze a wide range of organic pyrophosphates and has a 'housecleaning' function. The previous study showed that *Trichinella spiralis* Nd (TsNd) bound to intestinal epithelial cells (IECs), and vaccination of mice with rTsNd, TsNd DNA injected intramuscularly or *delivered by attenuated live Salmonella, produced a partial* protective immunity against *T. spiralis* infection. In this study, three TsNd specific small interfering RNA (siRNA) were designed to silence the expression of TsNd in *T. spiralis* larvae.

SiRNAs were delivered to the larvae by electroporation. Silencing effect of TsNd transcription and expression was determined by real–time PCR and Western blotting, respectively. The infectivity of the larvae treated with siRNA was investigated by the in vitro larval invasion of IECs and experimental infection in mice.

The results showed that siRNAs were efficiently delivered into *T. spiralis* larvae through electroporation. Real–time PCR and Western blotting showed that transcription and expression level of TsNd gene was inhibited 73.3 % and 76.7 % ($P < 0.05$), respectively, after being electroporated with 2 μM of siRNA–275 for 1d. Silencing TsNd expression inhibited significantly the larval invasion of IECs in a dose–dependent manner (r =–0.97941). The invasion rate of the larvae eletroporated with 1, 1.5, 2, 2.5 or 3μM siRNA–275 for 18 hours were 50.3 %, 42.6 %, 37.0 %, 33.6 % and 30.6 %, respectively, while the invasion rate of the larvae treated with control siRNA and untreated group was 59.4 % and 58.3 % ($P < 0.05$), respectively. The mice infected with larvae treated with TsNd siRNA displayed a 63.6 % reduction in adult burden and 68.8 % reduction in muscle larval burden compared with mice infected with control siRNA treated larvae ($P < 0.05$).

Our results showed that silencing TsNd expression in *T. spiralis* significantly reduced the larval infectivity and survival in host, further indicating that TsNd plays an important role during *T. spiralis* larval invasion, development and survival in host.

Host–Pathogen-Interaction and Immunology

Effect of probiotic bacteria on phagocytosis and respiratory burst activity of blood polymorphonuclear leukocytes in mice infected with *Trichinella spiralis*

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Probiotic strains confer a beneficial property to the host as immune stimulation, protection against pathogens and have the capacity to control intestinal parasite infection but also some nongut infection. Phagocytosis and respiratory burst are two of the most important functions of leukocytes and essential for the elimination of invading pathogens. This study focused on the effect of probiotic (bacteriocinogenic) strains on parasite infection and innate immunity (phagocytosis and oxidative burst of PMNL) in mice infected with *Trichinella spiralis.*

Bacteriocinogenic and probiotic strains of different origin (*Enterococcus faecium* AL41, *Enterococcus durans* ED26E/7, *Lactobacillus fermentum* AD1–CCM7421, *Lactobacillus plantarum* 17L/1) were administered daily at a dose of 10^9cfu/ml in 100µl. Mice were infected with 400 larvae of *T. spiralis* on the 7th day of Treatment.

The results indicate that phagocytic and metabolic activities of blood leukocytes are inhibited at weeks 3 and 4 post *T. spiralis* infection, i.e. in the time of massive blood migration of newborn larvae into the muscles. Administration of bacterial strains to mice with *T. spiralis* infection stimulated phagocytic activity of leukocytes and their ingestion capability from 1 to 3 weeks of the infection and the phagocytosis was inhibited only at week 4 p.i. The highest stimulative effect on phagocytosis was induced by strains *E. durans* ED26E/7 and *L. plantarum* 17L/1. The percentage of cells with respiratory burst and their enzymatic activity were increased after *T. spiralis* infection with the exception of week 3 p.i. But in mice treated with bacterial strains the enzymatic stimulation was observed for all time after the infection, with the highest intensity caused by strains *E. durans* ED26E/7, *L. fermentum* AD1–CCM7421 and *L. plantarum* 17L/1.

The administration of probiotic strains stimulated phagocytosis and respiratory burst of blood leukocytes that could contribute to a decreased larval migration and a destruction of newborn larvae and then reduced parasite burden in the host. The highest protective effect against *T. spiralis* infection was induced by strains *E. durans* ED26E/7 and *L. plantarum* 17L/1.

Host–Pathogen-Interaction and Immunology

Identification of Th2 epitope of paramyosin from *Trichinella spiralis*

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Trichinellosis is a widespread zoonosis. Paramyosin from *Trichinella spiralis* (*Ts*–Pmy) has been proved to be a good vaccine candidate against trichinellosis according to our previous work. As humoral immunity plays an important role in the elimination of the parasite, identification of Th2 epitope of paramyosin is crucial to construct the chimeric Th–B epitope vaccine. Based on BALB/c mice model, H–2d restricted Th epitopes of Ts–Pmy were predicted by SYFPEITHI database.

Candidate epitope peptides were synthesized by solid–phase procedure. BALB/c mice were immunized with recombinant *Ts*–Pmy (r*Ts*–Pmy) and the candidate epitope peptides. The polarized directions stimulated by candidate peptides were verified by cytokine production. Twelve Th epitope peptides with the highest score were determined by bioinformatics approaches. Stimulated by candidate peptides P2, P3, P4 and P5, Th2 type cytokines (IL–4 and IL–5), were increased in the splenocyte of the mice immunized by r*Ts*–Pmy and respective epitope peptides.

These four peptides stimulated significantly higher T cell proliferation by $CD4^+$ lymphocyte proliferation assays. Th epitope of P2, P3, P4 and P5, which could induce Th2 immune responses, are Th2 epitopes of *Ts*–Pmy. This research laid a foundation for designing effective chimeric Th–B epitope vaccine against trichinellosis

Host–Pathogen-Interaction and Immunology

Regulatory parameters of the lung immune response during the early phase of experimental trichinellosis

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Parasitic infection caused by *Trichinella spiralis* early stimulates the mucosal immune system at the intestinal level which causes an allergic inflammatory response in the lungs. We have demonstrated in Wistar rats that this process is characterized by humoral, cellular and functional changes which are finally biased towards a Th2–type immune response. Considering that helminth parasites are widely recognized as masterful regulators of their host's immune response, this study was aimed to: I) detect the presence of regulatory parameters at the lung level during the early phase of infection; II) analyze the ability of newborn larvae (NBL) to modulate the activation of macrophages from the lung parenchyma.

I) The kinetics of the emergence of regulatory T cells (Tregs), alternatively–activated macrophages, TGF–β and IL–10 were measured by flow cytometry, by determining arginase activity, and by ELISA respectively. II) In *in vitro* assays, macrophages from the lung parenchyma (MØ) of non–infected or of day 6 post–infection (pi) rats were cultured with live NBL (NBLl) or dead NBL (NBLd). After 48 hours, arginase activity was measured in cell lysates (alternative activation of MØ) and the production of nitric oxide (NO) was measured by assaying nitrites in the culture supernatants using the Griess reaction (classical activation of MØ).

Our results revealed a significant increase in: I) the % of Tregs at days 6 and 13 pi ($P < 0.01$); the levels of arginase activity at day 13 pi ($P < 0.05$); IL–10 levels between days 3 and 6 pi ($P < 0.01$); and TGF–β levels between days 6 and 13 pi ($P < 0.01$). II) Only NBLl were able to cause a significant increase in the levels of arginase activity and NO ($P < 0.05$) in MØ of non–infected rats. NBLl and NBld were able to cause a significant increase in the levels of arginase activity ($P < 0.05$) in MØ of day 6 pi rats. Only NBLl were able to cause a significant increase in the levels of NO ($P < 0.05$) in these MØ.

These results show the emergence of a regulation of the lung immune response during the early phase of infection that could be associated with the passage of NBL through that organ. For the first time the ability of NBL to modulate the activation of macrophages from the lung parenchyma, through excretion–secretion products and/or parasitic cuticle, is shown.

Host–Pathogen-Interaction and Immunology

Cysteine peptidase inhibitors of *Trichinella spiralis*

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Trichinella spriralis is well known causative agent of human trichinellosis. Individuals of this nematode parasite produce various types of cysteine peptidase inhibitors (cystatins) in order to regulate the function of cysteine peptidases and modulate the immune response of the host organism. Therefore we aim to reveal the differences in production of cystatin by different *T. spiralis* developmental stages.

Three types of cystatins were identified in the genome of *T. spiralis* – cystatin B, onchocystatin and multi cystatin domain protein. ICR and SCID mice were experimentally infected *per os* with infective muscle stage larvae (ML) of *T. spiralis*. Preadults (Ad3), adults (Ad5), new born larvae (NBL) and muscle stage (ML) larvae of *T. spiralis* were isolated from the intestinal and muscle tissue at various time post infection. Excretory/secretory products of all developmental stages were isolated and screened for the presence of cystatins using mass spectrometry. The cDNA was reversely transcribed from RNA of all stages and genes for cystatins were amplified using specific primers. Production of three types of cystatins by all studied stages was analyzed and evaluated.

Host–Pathogen-Interaction and Immunology

Biochemical alterations of host environment can modulate experimental *Trichinella spiralis* infection

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The host–parasite interaction can be altered by changes in the host environment that may or may not be in favor of successful invasion by the nematode parasite *Trichinella spiralis*. Metformin and atorvastatin are applied on a wide scale, to the degree that they could be considered as part of the host biochemical environment that can affect the parasite. Therefore, this study aimed to investigate the impact of alteration of the host's biochemical environment by these commonly used drugs upon the course of *T. spiralis* infection.

Mice were infected with *T. spiralis* then divided into three groups: (1) received atorvastatin, (2) received metformin, and (3) untreated. From each group, small intestines and muscles were removed for histopathological and biochemical analysis as well as total muscle larval counts.

We found that the total larval counts in muscles as well as the oxidative stress and the expression of vascular endothelial growth factor in the muscles were significantly reduced in both drug–receiving groups as compared to the infected control group. Moreover, marked reduction in the inflammatory cellular infiltration, cyclooxygenase–2 expression, and oxidative stress was noted in the small intestines of the treated groups as compared to the infected control group.

In conclusion, this study provides us with many insights about different biochemical changes in the host that the parasite has to face. Moreover, the anti–inflammatory and anti–angiogenic effects should be taken into consideration when treating infections in patients on therapy with atorvastatin or metformin.

Host–Pathogen-Interaction and Immunology

Modulation of lymphocyte populations by cornelian cherry *(Cornus mas L.*) active compounds in mice infected with *Trichinella spiralis*

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Cornelian cherry *(Cornus mas L.*) is a widely used plant in traditional European and Asiatic medicine. The high level of physiological and therapeutic activities of *C. mas* fruits are caused by the presence of biologically active compounds, such as vitamin C, organic acids, pectins, phenolic acids, flavonoids (anthocyanins, flavonols), triterpenoid (ursolic acid) and iridoids (loganic acid, cornuside). Anthocyanins are known as modifiers of inflammatory processes having antitumor and antioxidative properties. Iridoids, among which the most abundant are loganic acid and cornuside, exhibit different pharmacological properties including anti–inflammatory or hypotensive activity. The aim of this study was to investigate the influence of iridoids and anthocyanins of cornelian cherry fruits on the immune response during the intestinal phase of *T. spiralis* infection.

Balb/c mice were infected with 200 larvae of *T. spiralis/*mouse. Iridoids and anthocyanins aqueous extract of cornelian cherry fruits (100 mg/kg b.w.) was administered orally for 6 days, between 3th day prior to infection and 3th day after infection (dai) with *T. spiralis*. The control group consisted of mice infected with *T. spiralis*. The lymphocytes were obtained from the spleen and mesenteric lymph nodes (MLN) on 5th, 7th, 14th, 21th dai and T lymphocyte subpopulations (CD3$^+$, CD4$^+$, CD8$^+$ cells) and B lymphocyte population (CD19$^+$) were analyzed by flow cytometry. The CD4$^+$/CD8$^+$ lymphocyte ratio of spleen and MLN was also calculated. The parasite loads in the intestine were determined on 5th, 7th, 14^{th,} 21th dai.

Iridoids and anthocyanins aqueous extract exerted the modulating effect on the subpopulation of CD4$^+$ and CD8$^+$lymphocytes in the spleen and MLN. The changes in the percentage of CD3$^+$ lymphocytes were observed in the spleen. The CD4$^+$/CD8$^+$ ratio increased in the spleen on 5th *dai.* The significant increase in the percentage of CD4$^+$ lymphocyte was observed on 5th and 7th dai in the spleen and on 7th and 14th dai in MLN. The percentage of CD8$^+$ lymphocytes increased on 7th and 14th dai in the spleen and on 14th dai in MLN. The increase in the percentage of CD3+ splenocytes was noticed only on 5th dai, but B splenocytes population (CD19$^+$) decreased on 5th and 7th dai. Moreover, the number of adult *Trichinella spiralis* on 5th dai in mice receiving iridoids and anthocyanins extract was lower than in the control mice.

These results suggest that iridoids and anthocyanins aqueous extract of cornelian cherry fruits stimulate the murine cellular immune response during intestinal phase of *T. spiralis* infection.

Host–Pathogen-Interaction and Immunology

Application of microarrays to the analysis of Nitric Oxide pathway in monocytes of mice infected with *Trichinella spiralis*

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The aim of this study was to determine the NO (Nitric oxide) pathway gene expression profiles at 10, 20 and 39 days after *Trichinella spiralis* experimental infection in BALB/c mice. For this purpose, DNA microarray analysis was used that allows the measurement of the expression levels of a large number of genes simultaneously. Labeled cDNA from white blood cell total RNA was hybridized on a nylon membrane printed with 114 NO pathway gene–specific oligos. The analysis of each microarray was performed by GEArray Expression Analysis Suite 2.0 and BRB array tools.

Out of 114 genes, 18 (15,8 %) genes were present in non–infected and post–infected mice. The expression of Ilk and Mt2 genes was significantly upregulated 10 days post–infection, while the expression of Mt2 gene was also significantly upregulated 20 days post–infection. Furthermore, the expression of Fos, Fth1, IL–1b and Nfkbia genes was significantly downregulated 10 days post–infection, while the expression of Cxcl2 gene was significantly downregulated 10 and 20 days post–infection.

Further investigation of these genes is necessary in order to elucidate the *T. spiralis* modulation mechanism of the Nitric Oxide signaling pathway.

Host–Pathogen-Interaction and Immunology

Inhibitory effect on BALB/c nude mice bearing human H7402 solid tumor by administrated the A200711 protein from *Trichinella spiralis*

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To investigate the inhibitory effect on tumor–bearing mice treated with A200711 protein from *Trichinella spiralis*, human hepatocellular carcinoma H7402 cells were grafted in back of BALB/c nude mice.

After bearing tumor, the mice were treated at 7.5μg/mL A200711 protein every two days. The growth, diameter of solid tumor and weight of tumor bearing mice were recorded to calculate the tumor volume and draw the tumor growth curve every 3 days. After 3 weeks, the tumor–bearing mice were sacrificed. Tumors were harvested, weighted and calculated for the inhibitory rate. After 20 days, all experimental nude mice beared tumors. The tumor grafted rate was 100 %, the average diameter was 3–5mm.

Compared with the model control group, the solid tumor volume of nude mice treated with the A200711 protein was drastically shrunk and the inhibitory rate reached 39.67 % after 12 days. The A200711 protein from *T. spiralis* significantly inhibited the expansion of human hepatocellular carcinoma H7402 cells in nude mice.

[§]These authors contributed equally to the work.

Host–Pathogen-Interaction and Immunology

Cloning and immunological identification of the 14–3–3 protein from *Trichinella spiralis*

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Trichinellosis, a widespread zoonosis, is regarded as an emerging or re–emerging disease. The development of a vaccine to prevent infection by *Trichinella* in domestic animals and humans is a necessary approach for controlling this disease.

The 14–3–3 is not only a family of highly conserved proteins in various species but also is a promising prospect as a target molecule for early diagonosis and vaccine candidate antigen. In our previous study, *Ts*–14–3–3 was identified by infection sera from swine or mice infected with *T. spiralis* using immunoproteomic profile.

The recombinant protein (r*Ts*–14–3–3) was expressed and purified by Ni–affinity chromatography. Western blot showed that the r*Ts*–14–3–3 could be recognized by sera from human patients, *T. spiralis* infected swine, rabbits and mice, respectively. Immunolocalization demonstrated that *Ts*–14–3–3 was rich on the surface of *T. spiralis* muscle larvae. The expression of *Ts*–14–3–3 was detected in both the adult and muscle larval stages at the mRNA and protein expression levels.

BALB/c mice vaccinated with r*Ts*–14–3–3 demonstrated 43.9 % reduction in muscle larvae burden and 34.5 % rate of adult worm reduction burden following *T. spiralis* larvae challenge. Vaccination of the mice with r*Ts*–14–3–3 resulted in high level of specific anti–*Ts*–14–3–3 IgG antibodies and generated a Th1/Th2 mixed type of immune responses dominated by Th2. The present results indicate that *Ts*–14–3–3 is a possible candidate vaccine against *T. spiralis* infection.

Host–Pathogen-Interaction and Immunology

Characterization and functional analysis of *Trichinella spiralis* Nudix hydrolase

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Trichinella spiralis Nudix hydrolase (TsNd) was identified by screening a T7 phage display cDNA library from *T. spiralis* intestinal infective larvae (IIL), and vaccination of mice with recombinant TsNd protein (rTsNd) produced a partial protective immunity.

The aim of this study was to identify the characteristics and biological functions of TsNd in the process of invasion and development of *T. spiralis* larvae. Transcription and expression of TsNd gene at all developmental stages of *T. spiralis* were observed by qPCR and immunofluorescent test (IFT). The rTsNd had enzymatic activity to dGTP, NAD, NADP and CoA.

Its kinetic properties on the preferred substrate dGTP were calculated, and the V_{max}, K_m, and k_{cat}/K_m values at pH 8.0 were 3.19 $\mu M \cdot min^{-1} \cdot \mu g^{-1}$, 370 μM, and 144 $s^{-1} \cdot M^{-1}$, respectively, in reaction matrix containing 5 mM Zn^{2+} and 2 mM DTT. The rTsNd was active from 25 to 50°C, with optimal activity at 37°C. rTsNd was able to bind specifically to mouse intestinal epithelial cells (IECs) and promoted the larval invasion of IECs, whereas anti–rTsNd antibodies inhibited the larval invasion of IECs in a dose–dependent manner.

Anti–rTsNd antibodies could kill *T. spiralis* infective larvae by an ADCC–mediated mechanism. The results showed that the rTsNd protein was able to interact with host IECs and had the Nudix hydrolasing activity, and the enzymatic activity appeared to be essential indispensable for the *T. spiralis* larval invasion, development and survival in host.

4.4 Detection

Prokaryotic expression and reactivity analysis of serine proteinase inhibitor gene of *Trichinella spiralis*

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The specific primers derived from *T. spiralis* serine proteinase inhibitor (TsSerPIN) gene in GenBank database were designed and used to amplify the TsSerPIN gene from total RNA isolated from *T. spiralis* muscle larvae. The RT–PCR product was ligated into the expression vector pET30a and recombinant plasmid was transformed to *E. coli* BL21 (DE3) and induced by IPTG for expression.

The results showed that pET30a–TsSerPIN1 recombinant expression plasmid contained an insert of 1122 bp encoding 373 amino acids which showed 99 % identity to TsSerPIN gene in GenBank database. The TsSerPIN1 recombinant protein was highly expressed in the form of inclusion body with the molecular weight of about 48.5 kDa. In Western–blot analysis the purified TsSerPIN1 recombinant protein could be specifically recognized by *T. spiralis* infected swine serum. Different fragments of the TsSerPIN gene were further cloned and expressed in *E. coli* system.

Western–blots indicated that TsSerPIN1, TsSerPIN2 (159–346 aa) and TsSerPIN3 (309–373 aa) recombinant proteins could be recognized by *T. spiralis* infected swine serum. However, TsSerPIN4 (309–373 aa) and TsSerPIN5 (159–318 aa) could not combine to the swine serum that infected with *T. spiralis*. The results indicated that the main epitopes were disturbed between 257 aa and 346 aa of TsSerPIN protein, which would be used for further diagnosis of *T. spiralis* infection.

Detection

Antibodies dynamics of mice infected with *Trichinella spiralis*

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Excretory–secretory antigens (ES) produced by 3–day–old adult worms (AD3), 6–day–old adult worms/newborn larvae (AD6 + NBL) and muscle larvae (ML) of *Trichinella spiralis* were tested in the study. Mice were inoculated with muscle larvae of *T. spiralis* (ISS534). To assay the antibody response by drawing the antibody dynamic curves, the tail vein blood of infected mice was collected at different days post infection (dpi) within five months and separated serum for measuring the IgM and IgG by ELISA method.

The results indicated that ML ES antigens could detect the anti–*Trichinella* IgM and IgG at 5 and 20 dpi, respectively. The IgM and IgG could be detected by ES antigens of AD3 and AD6+NBL at 8 and 15 dpi. In regard to dynamics of anti–*Trichinella* antibodies of mice infected with *T. spiralis*, the most interesting finding was that AD3 and AD6+NBL ES antigens could be also recognized by early infection serum.

These antigens may be good candidates for the early diagnosis of trichinellosis, because antigens from adult and newborn larvae do not have glycosylation, which may reduce the cross–reaction produced by ES of ML in the immunodiagnosis of trichinellosis due to ES of ML glycosylated with tyvelose.

[§]These authors contributed equally to the work.

Detection

New strategies for improving the serodiagnosis of *Trichinella* infection in pigs

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Excretory–secretory (E–S) proteins produced by first stage larvae (L1) of *Trichinella spiralis* are commonly used as antigen in indirect ELISA for ante–mortem screening of pigs for *Trichinella* infection. Several factors can seriously affect the performance of this assay, including the conditions of such steps as *in vitro* incubation of L1, processing of the E–S antigen–containing culture medium, and storage of the antigen.

Moreover, it is difficult to establish the nature of cross–reacting antibodies due to the complexity of E–S antigen, which consists of multiple proteins many of which are represented by different isoforms. Although a set of immunodominant E–S proteins is reproducibly present in every preparation of the antigen, there is evidence of batch–to–batch variation in its total protein composition. To preserve the quality of E–S antigen, we ensured its stability during post–harvest processing and storage.

This was achieved by maintaining the E–S antigen–containing culture medium at low temperature and supplementing it with protease inhibitors immediately after clarification, followed by transferring the antigen from culture medium to an appropriately formulated storage buffer containing protease inhibitors. To ensure reliable performance of the assay each step of the ELISA protocol was evaluated and optimized. The revised protocol resulted in 99.8 % specificity (% PP cut off of 16 %) when sera from 1000 sows were tested. Nevertheless, this suggests that confirmatory testing may still be necessary to rule out false positive results.

To further reduce the likelihood of non–specific reactivity in the ELISA, we are pursuing new strategies, including selective coating of ELISA plates with only the immunodominant constituents of the E–S protein complex, using capture monoclonal antibodies specific to the TSL–1 group epitope of *Trichinella*. Another approach is the production of specific components of the E–S protein complex of *Trichinella spiralis* in the yeast expression system, *Pichia pastoris*, for use as recombinant antigen.

Detection

Validation of the Trichin–L Antigen Test Kit for the detection of *Trichinella* larvae in meat products

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Human trichinellosis is a foodborne disease caused by ingestion of meat infected with *Trichinella* muscle larvae. To control *Trichinella* spp. infection in the European Union, slaughtered pigs and other animals susceptible to *Trichinella* infection and intended for human consumption should be examined by one of the approved digestion methods described in Regulation (EC) No. 2075/2005. Traditionally, the magnetic stirrer method is considered as the gold standard for the detection of *Trichinella* larvae in fresh meat. However, this method is very laborious, subjective and has a low sensitivity of 3–5 larvae per gram (LpG). Recently, a new, non–microscopic method (Trichin–L) was developed which is based on the detection of *Trichinella* antigen using monoclonal antibodies and enables an objective evaluation of the results. The method has been approved as an alternative to the magnetic stirrer method for the detection of *Trichinella* in fresh meat.

Cured meat products are popular in many parts of the world, but curing and smoking processes are not recommended for control of *Trichinella*. In the past years, *Trichinella* outbreaks due to the consumption of cured wild boar or pork products have been described in Germany, making the identification of the larvae from these products relevant for *Trichinella* control. Therefore, this study aimed to validate the *Trichinella* Antigen Test Kit (Biorad) for routine testing of cured meat products.

The test was validated according to OIE Guidelines using spiked pork samples and cured pork products. In fresh pork, the Trichin–L achieved comparable analytical and diagnostic sensitivity as the gold standard.

The detection rate reached 100 % for 3 lpg and >90 % for 1 lpg in fresh pork, though a susceptibility of the Trichin–L test to cross–contamination with *Trichinella* antigen was revealed. A detection rate of 80 % for 3 lpg and 50 % for 1 lpg was found in smoked pork ham. For 3 lpg in salami, bacon and ham 20 %, 60 % and 100 % sensitivity were achieved, respectively. Further, to determine the performance of the test under field conditions, pork products from regions with known high *Trichinella* prevalences confiscated by customs authorities at international German airports were analyzed. Problems associated with the Trichin–L test were incomplete digestion due to fatty ingredients, spices and very dry meat products, resulting in data which could not be evaluated.

Therefore, the test is currently not suitable for the detection of *Trichinella* larvae in meat products and needs further adaptation steps to increase both sensitivity and specificity.

4.5 Epidemiology

Endemic sylvatic trichinosis in Abruzzi region (Central Italy) and the epidemiological role of the wolf

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The Abruzzi is a region of Central Italy with more than one third of its territory covered by national parks and protected nature reserves. It harbours one of the most consistent wolf populations in Italy. Since 2004, the Istituto Zooprofilattico Sperimentale Abruzzo and Molise (IZSAM), has performed tests for the detection of *Trichinella* larvae according to the EU Regulation EC 2075/2005. In this framework, all the wild species that can maintain the parasite in a sylvatic cycle are routinely sampled and tested according to the internal necropsy procedures. Testing results are registered in an automatized data collection system developed by IZSAM where all information regarding the health status of wildlife are stored and analysed.

Both magnetic stirrer and Trichomatic™ methods were used during the 2004–2014 period. Overall, more than 135,000 tests were carried out during the period, ranging from 1,090 to 20,897 (average 12,307 tests/year), prevalently by magnetic stirrer method (57.8 %), of which 117,024 were swine diaphragmatic muscles (86.4 %) and 18,351 were wildlife samples (13.6 %).

Not one positive sample was detected in the swine species. In contrast, 91 positive samples were detected from six wild species, namely the wolf (*Canis lupus*, 59 positives out of 218 samples), the fox (*Vulpes vulpes*, 24/480), the wild boar (*Sus scrofa*, 3/16,323), the beech marten (*Martes foina*, 2/27), the pine marten (*Martes martes*, 2/6) and the wildcat (*Felis silvestris*, 1/8). All isolates submitted to the National Reference Center for identification by multiplex PCR resulted to belong to the *Trichinella britovi* (T3) species.

According to the EU regulation a monitoring plan on wildlife must be carried out in the regions when applying for *Trichinella* free status for a pig herd. The collection of information on factors that can favour the circulation of *Trichinella* spp. in nature is fundamental to assess and manage the risk for domestic animal. The red fox is considered the main reservoir of *T. britovi*. However in the last decades in Central Italy the wolf population has increased significantly and its number is continually growing. Therefore, the epidemiological role of the wolf as possible indicator of trichinosis infection in nature should be reconsidered in some areas, considering likewise this species, in the *T. britovi* monitoring.

Epidemiology

Trichinellosis in wolves (*Canis lupus*) in Poland

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In Poland both the sylvatic and domestic cycles of *Trichinella* are known. Domestic trichinellosis occur in pigs, horses, and man. The sylvatic cycle involves infection by wild boars, foxes, other carnivorous and omnivorous species such as wolves, raccoon dogs, lynxes, badgers, martens and small rodents, which enables the maintenance and dissemination of the infection in the wild on Polish territory. It is known, that wild carnivores, such as wolves or foxes, migrate over great distances and their carcasses can become excellent fodder for other carnivorous animals and wild boars when they are shot or die. Although carnivores are involved in the circulation of the *Trichinella* nematode in the natural environment it is difficult to estimate their real role in the cycle due to their populations being small and often legally protected. In Poland, the legal protection of wolves was implemented in 1998. Now, there are about 900–1000 wolves in Poland. The majority of the Polish wolf population is a vital part of a continuous Eastern Europe wolf population and inhabits the north–eastern, eastern and southern part of Poland. The biggest refuge for Polish wolves is the Carpathian Mountains. This population is partially shared with the Slovakia and Ukraine. The other main wolf areas are the large forest complex of north–eastern Poland. Isolated populations in Western Poland, near the German–Polish border comprise only a few individuals. The aim of the study was to investigate the prevalence of *Trichinella* infection in wolves in Poland.

Muscle samples (diaphragm pillars, muscles of paws and hind legs) from 22 hunted and perished wolves were collected between 1999 and 2015 in two regions: Bieszczady Mountains and Augustowska Forest. The weight of the examined muscle samples varied from 5.5 g to 106.32 g. Muscle samples were digested individually using a standard protocol. Larvae were counted and the intensity of infection was calculated as the number of larvae per gram (LPG) of muscle tissue. Muscle larvae were detected in 12 wolves (54.5 %) and identified at species level by multiplex PCR. *T. britovi* was determined in all positive wolves. The infected wolves harbored from 0.009 to 27 LPG.

Our results confirm that wolves play a key role in maintenance of *T. britovi* nematodes through the sylvatic cycle. However, the wolf is not recognized as the direct source of *Trichinella* cases, but its carcasses could be a possible source of contamination for other animals.

Epidemiology

Investigation on the genetic structure of *Trichinella spiralis* from pigs, rats and wild boar of Poland

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The nematode *Trichinella spiralis*, the most important etiological agent of trichinellosis in humans, shows a cosmopolitan distribution, and it is sometimes detected in backyard pigs and hunted wild boar (*Sus scrofa*). In the course of human and animal outbreaks, it is extremely difficult to trace the origin of the infection, as the *Trichinella* species identification does not provide sufficient data to recognize the infection source.

The aims of this study were to investigate the genetic structure of *T. spiralis* of Poland using microsatellite markers. To this end, isolates from 3 backyard pigs and one synanthropic rat (*Rattus norvegicus*) were collected from a single Polish farm, and isolates from 11 wild boars hunted in 7 different Polish regions were also included. From each of the 15 *T. spiralis* isolates, multiple larvae (from 9 to 36) were tested and analyzed individually. Out of nine microsatellite loci investigated, six were polymorphic and were retained for genetic analysis. Allele sizes were determined by capillary electrophoresis of amplification products. Allele and genotypic frequencies were analyzed using the GenePop 4.0 and GenAlEx 6.501 software. Phenotypic analysis based on presence/absence of alleles was performed using multivariate analysis by Past 3.02 software.

Genetic variability at the population level was measured by (i) the number of alleles per locus; (ii) the percentage of polymorphic loci; and (iii) the observed/expected heterozygosity. The analysis shows a low level of genetic variability in the 15 *T. spiralis* Polish isolates. Overall, the analysis shows a low level of genetic variability, but a high genetic differentiation in the 15 *T. spiralis* Polish isolates. In fact, a wide range value (0.006 to 0.956) was observed among the wild boar isolates by the Wright's Fixation (Fst) index; whereas, the isolates from the 3 pigs and the rat from the single farm show low Fst values (range 0.000–0.050). However, it was noticed a poor correlation between the genetic differentiation and the geographical distance of wild boar isolates.

The multidimensional space based on phenotypic data drawn by the first three coordinates (taking in to account the 78 % of the variance of the variable distribution) confirms the genetic results. This study represents the first attempt to investigate the transmission of *T. spiralis* at the farm level and provides novel information on the genetic structure of *T. spiralis* populations circulating in Poland.

Epidemiology

Trichinellosis in wild boars in the Czech Republic

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Trichinellosis is a food borne zoonotic disease caused by the consumption of raw meat and raw meat–derived products from animals infected with nematode worms of the genus *Trichinella*. In Europe, the parasite is more prevalent in wildlife than in farmed animals and wildlife animals serve as the major reservoir hosts.

Between the years 2001 and 2015 (April), almost 1,7 million wild boars were hunted and of them 1,177 million wild boars (*Sus scrofa*) (69,2 %) were tested for *Trichinella* sp. in the Czech Republic and *Trichinella* infection was found in 18 wild boars (0,0015 %). Although the prevalence of *Trichinella* infection in wild boars is very low, the spatial analysis reveals that the level of risk differs by region in the Czech Republic. Larvae from wild boars were identified as *T. britovi* (66,6 %), *T. spiralis* (5,55 %) and *T. pseudospiralis* (22,22 %); one isolate (5,55 %) was without species identification; no mixed infection was not found.

In December 2010 and January 2011, *T. pseudospiralis* larvae were detected in three wild boars hunted in the eastern part of the Czech Republic. These *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 is probably 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 *Trichinella* larvae in wild boar meat samples.

Epidemiology

Trichinelosis in wild boar in Croatia (2010–2014)

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Wild boar hunting is a quite widespread activity throughout Croatia. At the same time, insufficiently thermally processed wild boar meat has been recorded in Europe as a source of trichinellosis in humans. Despite some sporadic cases and epidemics of human trichinellosis have been speculated by media to have originated from infected wild boar meat, no case of human trichinellosis caused by wild boar meat consumption has ever been published or expertly proven in Croatia. The aim of this work was to obtain data about the prevalence of *Trichinella* spp. in wild boar population, to identify the *Trichinella* species circulating in the Croatian territory and to estimate the potential risk of infection for humans in specific regions of Croatia on the basis of the information obtained from a five–year period of surveillance.

Inspection for *Trichinella* of shot wild boar carcasses in Croatia is mandatory since 1989. On the basis of the official information provided to the Ministry of Agriculture – Directorate of Veterinary Services in Croatia, in the 2010–2014 period 223 out of a total of 100,744 (0.22 %) wild boar meat samples were found positive for *Trichinella* spp. The results have been statistically processed.

Trichinella spp. infected wild boars were found in 17 out of 21 counties in which the Croatian territory is administratively divided. Nine wild boar meat samples have been submitted to the National Reference Laboratory for further examination. The intensity of infection in the muscles was 0.12–114.08 larvae per gram and involved *Trichinella* species were identified as *T. spiralis, T. britovi, T. spiralis+T. britovi*.

Epidemiology

Evaluation of the infectivity and the persistence of *Trichinella patagoniensis* in a new host, the guinea pig

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Trichinella patagoniensis is a new species of the genus *Trichinella* that is widespread in Argentina, with the cougar (*Puma concolor*) serving as a natural reservoir. Transmission of parasites of the genus *Trichinella* depends, among other factors, on the ability of muscle larvae to remain infective in muscle tissue under environmental conditions.

The aim of the present work was to study the infectivity and capability of *T. patagoniensis* muscle larvae to survive in decomposed muscle tissue of guinea pigs. Sixteen Ssi:AL guinea–pig–female were orally inoculated with 2000 muscle larvae (ML) of *T. patagoniensis* (ISS2311). After 42 days of infection, all animals were euthanized. Animals were eviscerated and corpses were placed on the surface of soil that was inside plastic boxes.

Boxes were subjected to the effects of weather conditions of the summer 2014–2015 in Buenos Aires, Argentina. At day 0, previous to the placing of the corpses, two infected animals were subjected to artificial digestion. Weekly, corpses were analysed by the same technique. In order to assess the infectivity of the ML recovered, 300 ML were orally inoculated to three female Balb/C mice.

After 42 days, mice were euthanized and the carcasses were digested. *Trichinella* ML were counted employing a stereomicroscope. The present study was approved under permit number 2014/01 by the Institutional Committee for Use and Care of Laboratory animals of the Faculty of Veterinary Sciences. Guinea pigs became infected with *T. patagoniensis,* ML were recovered from decaying muscle up to the third week of exposure to environmental conditions. ML became infective to Balb/C mice until the second week.

The RCI value in mice was reduced from 36.18 ± 3.44 to 15.3 ± 4.03 from week 0 to the second week. The maximum temperature recorded was 32 º C, the minimum temperature was 14.5 ºC with an average temperature of 26 º C and an average humidity of 67 %. Rainfalls were very frequent. The present results show for the first time that *T. patagoniensis* is able to complete its life cycle in guinea pigs, and thus, they could act as potential hosts of the parasite. *T. patagoniensis* would be able to survive and remain infective in rotten muscle for 2 weeks under the weather conditions of a hot summer, which would contribute to the maintenance of the sylvatic cycle of this parasite in the environment.

Epidemiology

Current results of the assessment of the prevalence of *Trichinella* spp. in red foxes (*Vulpes vulpes*) in the Western Alpine regions of Austria

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Within a recent study on the occurrence of zoonotical parasites in hunted red foxes (*Vulpes vulpes*) originating from Tyrol and Vorarlberg, we examined muscle samples for the presence of *Trichinella* larvae. This study was initiated to re–assess the epidemiological situation of *Trichinella* infection in Austrian carnivorous. Based on a statistical sample plan, 746 fox carcasses (Tyrol n=415; Vorarlberg n=331) were sampled during winter season 2013/14 and 2014/15. Data collection included the recording of the foxes' sex, age, weight, geographic origin of the samples and body condition. *Trichinella* examination was done by magnetic stirrer method for pooled samples. Muscle tissue (5 gram) of the front leg was digested and positive pooled samples were further individually tested in order to identify the infected animal. To determine the genotype of the recovered *Trichinella* larvae species differentiation was performed by multiplex PCR.

Trichinella larvae were observed in a total of 28 red foxes (3.2 %, with 95 %–confidence interval [1.5 %, 5.2 %]). In Vorarlberg an apparent prevalence of 6.3 % [3.9 %, 9.6 %] was observed, in Tyrol apparent prevalence was 1.7 % [0.6 %, 3.5 %]. Logistic regression indicates that the geographic origin of samples (province) and the age category have a significant effect on the likelihood of the occurrence of *Trichinella* larvae in the tested foxes. More specifically, red foxes originating from Vorarlberg have a significantly higher probability to be *Trichinella* positive compared to foxes from Tyrol (odds ratio = 4.1). Adult foxes are more likely infected compared to juvenile foxes (odds ratio = 2.7). Species identification from all positive samples revealed *Trichinella britovi* as the only infectious parasite species.

Our study confirmed that *Trichinella britovi* is prevalent in wild carnivores in Austria. In comparison to a previous study the present results revealed a higher prevalence in red foxes from Vorarlberg. In this province an increasing population of wild boars (*Sus scrofa*) is present. There is a potential risk of zoonotic transmission from red fox via wild boar to human. More attention should be given to the distribution and spread of wild boars especially into known *Trichinella* spp. infected areas. Therefore wildlife monitoring is an essential instrument to gain additional knowledge on the epidemiology of *Trichinella* spp. in Austria.

Epidemiology

First report of *Trichinella pseudospiralis* in Austrian wild boars (*Sus scrofa*)

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In Austria, more than 5 mio slaughtered fattening and breeding pigs are tested every year for *Trichinella* with no positive findings over decades. In wild boars the yearly hunting bag ranges between 20,000 and 30,000 animals. The major part of wild boars undergoes *Trichinella* examination by either artificial digestion method or trichinoscopy according to the Regulation (EC) No. 2075/2005. Findings of *Trichinella* in these wild animals are very rare and only single positive cases were documented in the past. As molecular diagnostic for species differentiation was not established at former time, the actual *Trichinella* species was unknown and all larvae were identified as *Trichinella spiralis.*

In the year 2011 and 2014 two cases of *Trichinella pseudospiralis* were detected in female wild boars which were hunted in the federal province of Styria and Burgenland. These two cases are the first reports of *Trichinella pseudospiralis* in Austria up to now. Both wild boars were tested in routine diagnostic by the magnetic stirrer method for pooled sample digestion. Species identification was done by multiplex–PCR.

In Austrian wildlife only *Trichinella britovi* is endemic and red foxes are the reservoir for these species. Findings are mainly located in Western provinces where also sporadic cases in badgers are documented.

Due to national legislation *Trichinella* testing of wild boars supplied from the hunter directly to the final consumer or to food businesses supplying directly to the final consumer is mandatory. In Austria *Trichinella* testing by using the trichinoscopic method is still allowed for direct marketing of wild boars. For food safety reasons, meat of these tested wild boars is prohibited to enter the production process in which *Trichinella* could survive. Trichinoscopy has a lower sensitivity for detecting *Trichinella* larvae in comparison to the digestion method. Therefore findings of non–encapsulated species are even more limited. The presence of *Trichinella pseudospiralis* is now confirmed in Austrian wildlife. As a consequence the further use of the trichinoscopic method for testing wild boars should be critically evaluated.

Epidemiology

Seroprevalence of *Trichinella* spp. in domestic dogs in Slovakia

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Trichinella spp., the causative agent of human and animal trichinellosis is widespread in wildlife of Slovakia. Red foxes and martens are the main reservoir animals and play a prime role in the maintenance of the *Trichinella*–sylvatic cycle. Moreover, urbanisation of these animals may represent an important risk for the parasite transmission into the domestic cycle (Hurníková et al., 2007, 2009).

Human trichinellosis is quite rare in Slovakia and only several infection outbreaks have been registered since the 1960ies (Reiterová et al., 2007; Paraličová et al., 2008). Nevertheless, the largest outbreak of trichinellosis in 1998 was associated with the consumption of dog meat. In total 336 persons were infected after consumption of smoked sausages in Valaská Village in central part of Slovakia (Dubinský et al., 2001). In this locality, traditional sausages are made from dogs´ meat only once a year during the traditional February folk festival. Only the above mentioned affair pointed out to an importance of this *Trichinella* infection source. Although dog meat consumption is not common in Slovakia, in certain minority ethnic groups of the Slovak population these eating habits have been confirmed. The aim of our study was to estimate the seroprevalence of *Trichinella* infection in domestic dogs in the territory of various regions of Slovakia.

Altogether 439 serum samples were tested using ELISA with *Trichinella spiralis* somatic antigen in dilution 1:200. Out of these, 56 dogs (12.8 %, CI 95 %: 9.7–15.9 %) were classified as seropositive. The highest seropositivity was recorded among dogs from the eastern part of Slovakia, in regions of Prešov (22.9 %) and Košice (18.9 %), which has been considered to be high endemic for *Trichinella* occurrence. Nevertheless, nearly 19 % seropositivity was also detected in dogs originating from the Trenčín region in north–western Slovakia. This area has been regarded as an endemic focus of *Trichinella* infection in the past ten years (Hurníková et al., 2009). In the rest of the country seropositivity for *Trichinella* spp. in dogs varied between 6 % and 12 %.

Presented results indicate that the dog population in Slovakia is at a high risk of exposure to *Trichinella* and can potentially serve as a source of the infection when used for uninspected food preparation.

Epidemiology

Trichinella parasite in invasive American mink (*Neovison vison*) in Poland

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The American mink (*Neovison vison*) is a semiaquatic mustelid species that originally comes from the North America. The first reports on American mink in Poland come from the early 50`s of the 20th century. By the end of the 90's it colonized almost the entire north and the central part of the country. Nowadays it inhabits nearly the entire country except the mountains and uplands of south–east Poland. Rapid colonization of new areas and strong competition with native predators identify the American mink as an invasive species. The aim of our study was to evaluate the role of the American mink in the circulation of *Trichinella* species in Poland.

In total 817 individual muscle samples obtained from dead or killed animals from National Parks and fur farms in different parts of Poland during 2008–2013 were examined for the presence of *Trichinella* muscle larvae using the artificial HCl–pepsin digestion method. Also specific *Trichinella* spp. antibodies in muscle juice samples were detected by indirect ELISA using excretory–secretory antigens of *T. spiralis* and *T. britovi*.

Muscle larvae were found in 27 out of 817 individuals (3.3 %) from national parks in western and central Poland. The intensity of infection varied from 0.1 to 274 LPG. The average seropositivity was much higher; specific *Trichinella* antibodies were detected in 18.57 % of investigated samples, suggesting the possible contact of the host with the parasite.

The results indicate that the carnivore is involved in maintaining *Trichinella* spp. in wildlife in Poland. The host–parasite relationships in individual national parks will be discussed with respect to habitats and diet of the American mink.

Epidemiology

Trichinella spp. in raccoon dogs (*Nyctereutes procynoides*) and red foxes (*Vulpes vulpes*) hunted in 2011–2012 in Estonia

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Raccoon dogs and red foxes are suitable indicator species and well–adapted hosts for *Trichinella* species circulating in Europe. In 2000–2002, *Trichinella* infections in these hosts were investigated in all Baltic counties, and 42.0 % raccoon dogs and 40.6 % red foxes that had been hunted in Estonia were infected with sylvatic *Trichinella* species. The aim of this study was to obtain updated data on *Trichinella* species circulating in raccoon dogs and red foxes in Estonia.

A nationwide sampling of the heads of raccoon dogs and red foxes was organized by the Estonian Veterinary and Food Board for evaluating the effectiveness of the wildlife oral vaccination program for rabies eradication. A subsample of these samples, in total 200 samples (113 raccoon dogs and 87 red foxes), was included in this study. Stratification by administrative units, based on the size of the areas, was used to obtain a geographically representative sample. From each animal, 20 grams of masseter muscle tissue was tested using an artificial digestion method. *Trichinella* species were identified using the multiplex–PCR method of the European Union Reference Laboratory.

The majority of animals were infected with *Trichinella* spp.: 57.5 % of raccoon dogs and 69.0 % of foxes. The parasite species identified were *Trichinella nativa* and *Trichinella britovi.* The prevalence was significantly higher than 10 years earlier in both host species.

Raccoon dogs and red foxes are relevant reservoirs for *Trichinella* spp. in Estonia and maintain a substantial infection pressure in the food chains, which include game animals and domestic animals intended for human consumption.

Epidemiology

The research of *Trichinella* prevalence of wild boars in areas affected by hunting

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Trichinosis in Latvia is a topical zoonosis. Within the project TCR / REP / 0065 (A) *"Meat product safety improvement in the Baltic region on the basis of trichinosis – parasitic zoonoses epidemiological control*" it has been found that the most common *Trichinella* species are *T.spiralis, T.britovi, T.nativa*. Trichinosis is common in the wild boar and is diagnosed across the whole territory of Latvia. The number of infested wild boars in different hunting areas is different. Our work objective was to compare the development of the *Trichinella* prevalence during a long period of time.

The study was carried out during the period of 2005 – 2014, in LUA Faculty of Veterinary Medicine, Food and Environmental Hygiene Institute laboratory of Parasitology. In total 2035 wild boar meat samples (diaphragm pillars sinewy parts) were examined. From Jelgava Municipality 507 samples of wild boar meat were sent, from Dobele Municipality – 504, from Talsi Municipality – 518 and from Ozolnieki Municipality – 506. Samples were examined using hydrolysis method (Regulation (EC) No.2075 / 2005).

The results showed that *Trichinella* infection was diagnosed in all municipalities involved in the research ($p < 0.05$). Following prevalence were found: In Dobele Municipality IE 4.4 %, in Jelgava Municipality IE 1.8 %, in Ozolnieki Municipality IE 1.2 % and in Talsi Municipality IE 0.9 %. In municipalities prevalences in boars differed in certain hunting areas.

Epidemiology

High prevalence of *Trichinella* spp. infection in carnivore mammals of Latvia

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Since the sixties, Latvia was known as a focus of *Trichinella* spp. infection in animals and humans with an average incidence of trichinellosis of 1.1 per 10^5 inhabitants in the last decade. The aims of the present work were to investigate the prevalence of *Trichinella* spp. infection in carnivorous mammals of Latvia, to identify the etiological agents, and to evaluate the worm burden, in a four year period (2011–2014).

Animal carcasses were provided by hunters. At least 25 g of muscles from the forelegs of each animal were tested by artificial digestion to detect *Trichinella* spp. larvae. Larvae were identified at the species level by multiplex PCR.

Out of 325 animals investigated so far, 207 (68.6 %) tested positive: 73/133, 54.9 %, pine marten *Martes martes*; 11/35, 31.4 %, stone marten *Martes foina*; 1/1 badger *Meles meles*; 3/3 lynx *Lynx lynx*; 72/95, 75.8 %, red fox *Vulpes vulpes*; 43/54, 79.6 %, raccoon dog *Nyctereutes procyonoides*; 4/4 wolf *Canis lupus*). The highest mean intensity of larvae (LPG) in the leg muscles was detected in raccoon dogs (23.9 LPG), followed by red foxes (21.03 LPG), stone martens (4.35 LPG) and pine martens (3.47 LPG). Most of *Trichinella* spp. larvae were identified as *T. britovi*. *Trichinella nativa* was rarely detected and always in mixed infections with *T. britovi*. *Trichinella spiralis* was identified in one stone marten in a mix infection with *T. britovi*.

These results confirm previous findings on the role of carnivorous mammals, canids in particular, as the most important reservoir species of *T. britovi* in Latvia. Since in the last decade, the estimated populations of red foxes and raccoon dogs have increased by 25 % and 50 %, respectively, we can speculate that the biomass of *T. britovi* in this country has increased sharply.

Epidemiology

Trichinella spp. in Northern Kazakhstan

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In Kazakhstan, approximately 0.58 trichinellosis cases per 100,000 inhabitants are reported each year, although substantial underreporting is expected. Most cases are thought to be associated with the consumption of undercooked meat from game and stray dogs. To date, there is not much data available on the prevalence of *Trichinella* in the region, but based on the *Trichinella* strains sent to the International *Trichinella* Reference Center, the parasite is most frequent in the sylvatic cycle, although infection in a domestic pig have been reported. *Trichinella* species found were predominantly *T. nativa* and *T. britovi*; hosts included the golden jackal, red foxes and wild cats.

Between October 2012 and March 2015, muscle tissue of 25 stray dogs, 20 stray cats, eight corsac foxes, three badgers, and 20 rats from different regions in the northern part of Kazakhstan (Akmola region, North Kazakhstan region, Karaganda region) were collected. Fifteen dogs, 20 cats, 20 rats, eight foxes and two badgers were examined from the Akmola region, 33 % of the dogs and all cats and rats originated from Astana city. Further, eight dogs and one badger were caught in the North Kazakhstan region and two dogs in the more southerly Karaganda region. 64 % (60 %) of the examined dogs (cats) were female and 36 % (40 %) male. Muscle samples were examined both by the magnetic stirrer and trichinoscopic method. The larval burden of the positive samples was determined and *Trichinella* species were identified by multiplex PCR and restriction fragment length polymorphism (RFLP).

Two of the three examined badgers were identified as *Trichinella* positive by both the magnetic stirrer and trichinoscopic method; one from the Kasilzar district in the North Kazakhstan region and one from Korgaldzin district in the Akmola region. In the badger from Kasilzar 70 larvae per gram of muscle tissue could be detected. The larvae were identified as *T. nativa* by both PCR and RFLP. The larval burden in the badger from the Akmola region was three larvae per gram; species identification was not performed. Four of the corsac foxes tested positive by trichinoscopic method. None of the examined stray dogs, stray cats and rats were infected with *Trichinella* spp.

The recent small outbreaks and these results show that public awareness of the risks associated with the consumption of raw or undercooked game meat in Kazakhstan must be heightened to increase consumer protection in the region.

Epidemiology

The occurrence of *Trichinella* spp. in respect to the gender of red foxes (*Vulpes vulpes*): preliminary results

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Trichinella nematodes are found in the domestic cycle in pigs, horses and humans, and in the sylvatic cycle in carnivorous and omnivorous animals such as wild boars, foxes, raccoon dogs, wolves and many others. It is known, that carnivores are involved in the circulation of nematodes of the genus *Trichinella* and they are a reservoir for trichinellosis in the natural environment. The red fox population in Poland has increased dynamically from 145,000 in 2000 to 213,000 in 2013. The increase is associated with the oral vaccination against rabies, easy adaptation to new habitats like suburban or urban areas and the use of human garbage as an easily accessible food. Red foxes migrate over long distances and their carcasses may be direct source of *Trichinella* infection for other carnivorous and omnivorous animals, such as wild boars and therefore indirectly increase the potential risk of human infection. The aim of this study was to determine the occurrence of nematodes of the genus *Trichinella* in red foxes in Poland.

Muscle samples (diaphragm pillars, tongues) were taken from 288 red foxes (120 females and 108 males) from the Głęboki Bród Forest District in north–east Poland (agreement NEU–0744 / LIFE–1/13/1 dated 02.18.2013, Project LIFE +) from 2013 to 2015. Samples were digested individually in HCl–pepsin solution. Larvae were identified as *Trichinella* based on morphology, than were counted and the intensity of infection was expressed as larvae per gram of muscle sample (LPG).

The infected foxes harbored 0.07 to 69 LPG. The weight of the muscle samples varied from 3.36g to 22.57g. The overall prevalence in all examined samples was found to be 28 % (64/228). *Trichinella* larvae were found in 30 % females (35/120) and 26 % males (29/108), however the prevalence varied between years. Genomic DNA was extracted from single larvae. Muscle larvae were identified at species level by multiplex polymerase chain reaction. Thirty–three females and twenty–five males were infected with *T. britovi*. One male was infected with *T. spiralis*. Five isolates were not identified probably due to DNA degradation. The presence of *Trichinella* nematodes was statistically analyzed with respect to sampling year and gender.

This study confirms that red foxes are involved in the maintaining of nematodes of the genus *Trichinella* in the wild in Poland. Additionally, the results of our research show that there are slight differences in infection by *Trichinella* in male and female red foxes, however further studies are necessary.

Epidemiology

Wild boars meat as a potential source of human *Trichinella* cases in Poland

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Trichinella nematodes circulate in domestic (pigs, horses and man) and wild animals (carnivores, omnivores). The sylvatic species most commonly infected with *Trichinella* are foxes and wild boars. *Trichinella* spp. may be transmitted between wild boars and red foxes via the ingestion of carcasses containing infective larvae.

The four species of *Trichinella*: *T. spiralis*, *T. native*, *T. britovi* and *T. pseudospiralis* have been confirmed to be present in several animal species in Poland. Trichinellosis is an epidemiological problem with a global distribution. In Poland a substantial increase of the wild boar population has been observed since 2010, together with an increased incidence of trichinellosis after ingestion of raw or undercooked wild boar products containing *Trichinella* spp. larvae. The actual number of human cases remains particularly difficult to determine. The aim of the present study was to determine the current prevalence and spread of these parasites within wild boars.

The diaphragm pillars and tongue from 834 wild boars were collected from 2010 to 2014 in different region in Poland. Additionally one wild boar meat sausage known to be a source of infection was examined. The samples were tested for *Trichinella* spp. using pepsin digestion. Total nucleic acid was isolated from individual larvae. DNA was isolated from a minimum of 10 larvae from each animal. Recovered larvae were identified at species level by multiplex polymerase chain reaction.

The overall prevalence in all examined samples was found to be 2.15 % (18/834). Recovered larvae were identified as *T. spiralis* and *T. britovi* (9/18 and 5/18, respectively). *T. spiralis* larvae were isolated from the sausage. Mixed infection was confirmed only once. Three isolates were not identified. The infected animals harbored 0.04 to 9.7 LPG.

The results of our study strongly confirm that the wild boars play a role in the maintenance of *Trichinella* nematodes through the sylvatic cycle. Everywhere the natural cycle exists, the real danger of the synanthropic cycle also exists, accompanied by a serious risk of human infection. This has been confirmed by our analysis of human outbreaks distribution in relation to the places where wild boar sausage was prepared.

Therefore, there is a clear need for the study of wild boar meat intended for human consumption due to the difficulties in detecting low–levels infections, as well as abnormal clinical symptoms during trichinosis.

Epidemiology

The peculiarities of trichinellosis epidemiology in the Arctic territories of the Far Eastern Federal District of Russia

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Main causative agent of trichinellosis on the Arctic coasts of the Russian Federation is *Trichinella nativa*. Trichinellids circulate in populations of predaceous animals in terrestrial and aquatic habitats, with involvement of synanthropic and pet animals, as well as fur animals which are kept in cages. It can be stated that some modes of traditional economic activity of indigenous people in Russian Arctic promotes the circulation of trichinellosis in this region. In addition to the human–related factors also feeding habits of local mammals as necrophagia, predations and cannibalism with the participation of numerous animals–disseminators are supporting the existence of trichinellosis nests.

The Arctic isolates of *Trichinella* are characterized by specific sets of traits enabling them to survive under low temperatures and other severe abiotic factors. Between main factors promoting the spread of trichinellosis in Russian Arctic is the regular consumption of un–cooked meat of wild animals by local indigenous people. It was experimentally demonstrated that even during the preparation of «kopal'chen» (fermentation of raw meat with putrefactive bacteria) *Trichinella nativa* juveniles retain their infectivity. Sixty–three (24.3 %) out of the examined 259 residents of coastal settlements were found to be immunopositive for *Trichinella* antigens. All the seropositive cases were represented by people belonging to three ethnic groups: Chukchi (97.8 %), Inuit (= Eskimos) (1.2 %), and Yakuts (1.0 %).

The antibody titers in ELISA varied from 1:100 (32.8 %) to 1:1600 (8.7 %). The highest titer reactivity was observed in marine mammal hunters, retired persons, and some groups of employees. There was a direct relationship between the antibody titer values and the dietary habits of the respondents with preference toward traditional food prepared from marine mammal meat. Thus, the monitoring and prevention of meat products involved into traditional life style of local population is between main applied aspects of trichinellosis control in Russian Arctic.

Epidemiology

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 trichinellae 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 *Trichinella* species, a large number of potential hosts and the existence of different life cycles. The sylvatic cycle is independent of human as natural hosts are related to wildlife population, in which trichinellae 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 of 627 foxes were investigated for the presence of larvae. In case of insufficient quantity of the primary sample, adequate quantities were assured by additionally/substitutionally investigation of the muscles of the upper forelimb.

The prevalence of *Trichinella* spp. larvae in foxes in Slovenia, calculated for the hunting season 2011/2012 on the basis of investigation of skeletal muscles, was 0.638 %.

Epidemiology

Trichinella britovi biomass in naturally infected pine martens (*Martes martes*)

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Parasites of the genus *Trichinella* are cosmopolitan nematodes infecting wild animals, which represent the main reservoirs of these zoonotic pathogens. To deeply investigate the helminth epidemiology in a geographical area, it is important to know their biomass. Attempts to evaluate the biomass in a host have been done for intestinal helminths by the egg count. No data is available on the biomass of *Trichinella* spp. larvae in muscles of naturally infected animals.

The aim of this work was to evaluate the larval biomass in naturally infected pine martens (*Martes martes*) of Latvia. Single muscles or group of muscles (abdomen, back, diaphragm, intercostal muscles, muscles from the head, left and right shoulders, lower and upper parts of the forelimbs and hind limbs, neck, rump with tail, and base and tip of the tongue), were entirely removed from the bones, and weighted from five (3 males and 2 females) skinned and eviscerated carcasses (average 1,065.8 g; range 740 g – 1,485 g) of *Trichinella britovi*–infected pine martens. The average muscle weight was of 488.5 g (range 322.8 g – 684.7 g). Each muscle or group of muscles was separately digested by HCl–pepsin to detect the number of larvae per gram (LPG).

The LPG ranking shows that the diaphragm had the highest number of LPG in all the five animals (average 56.93 LPG; range 5.07–117.31 LPG), followed by the lower part of forelimbs (average 44.25 LPG; range 4.28–86.8 LPG). The total larval biomass was of 52,644 larvae with an average of 10,529 larvae per animal (range 640–32,294). The group of muscles harboring the highest number of larvae was the neck (average 1,898 larvae; range 109–5,490 larvae). Using linear regression, the larval burden in each muscle or group of muscles was evaluated to measure the possible prediction of the total animal larval burden. All muscles were significantly predictive of the total burden (all adjusted R^2>0.78), and the left shoulder provided the highest adjusted R^2 (0.9981).

The assessment of the *Trichinella* biomass in nature can help to understand the epidemiological pattern of these pathogens, to implement actions aimed at controlling the infection in target animal species, and to acquire basic information on the complex biology of this group of zoonotic nematodes. This is the first attempt to investigate the biomass of *Trichinella* parasites and of *T. britovi* in particular, which is the most widespread species in Europe.

Epidemiology

The most important risk factors for domestic and sylvatic cycle of *Trichinella* species identified in an endemic district of Serbia

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Trichinella infection persists in Serbia and still causes serious human health, economic and social problems. Presence of *Trichinella species* was reported in domestic pigs and wildlife. Occurrence of human trichinellosis is attributed to a high prevalence of *Trichinella* infection in domestic animals, especially swine. The aim of the present study was to identify the risk factors for transmitting *Trichinella* spp. among domestic and sylvatic cycle in the endemic District Branicevo.

Trichinella infection was detected in domestic animals: pigs (*Sus scrofa domestica*) and dogs (*Canis lupus familiaris*), but also among wildlife in wild boars (*Sus scrofa*), red foxes (*Vulpes vulpes*), golden jackals (*Canis aureus*), wolves (*Canis lupus*). *Trichinella* infections in humans were related to cultural food practices, which include dishes based on raw or undercooked meat of domestic pigs and wild boar that were not subjected to the official veterinary control.

The Serbian national regulations for meat inspection and control differ from EU legislation and need to be up–dated. Epidemiological data presented in this paper show that risk factors for trichinellosis in domestic and sylvatic cycles are present in the endemic District Branicevo. For the domestic and synantropic cycles the following risk factors were recognized: husbandry conditions of backyard farms which are very poor due to intentional feeding of food waste containing pork scraps, scavenging of pigs in garbage dumps and improper disposing of pig carcasses in the field. Human actions such as the common habit of hunters leaving animal carcasses in the field have great influence on the sylvatic cycle.

The facts that *Trichinella* hosts express predominantly scavenger and cannibalistic behaviour, that stray dogs can access landfill dump and garbage as a food resource, indicate a high risk. GIS technology was used for mapping: epidemic events (*Trichinella* infection in animals and human, connections between human outbreaks and animal infection), as well as results of epizootiological surveillance (determination of infected animal species, investigation of endemic district) and results of scientific investigations (determination of *Trichinella* species in an examined area).

4.6 Human Trichinellosis and Treatment

Trichinellosis and ancient mummies

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The description by Ruffer in 1910 of *Schistosoma* eggs in an ancient Egyptian mummy was the beginning of numerous studies on the parasitological diseases affecting ancient populations. In their recent paper “Is atherosclerosis fundamental to human aging?

Lessons from ancient mummies”, Clarke et al, (2014) also mentioned the parasitological results obtained after the autopsy of the famous Nakht mummy and published again the picture of a *Trichinella* cyst (De Boni et al., 1977). This unique picture has led to the popular idea that trichinellosis was present in ancient Egypt as mentioned in nearly all reviews and PhD dissertation dealing with *Trichinella*. However, there is no convincing evidence that this cyst could be due to *Trichinella* as no worm remains are visible inside it (Dupouy–Camet, 2014).

Moreover, pork consumption in Ancient Egypt was probably infrequent as pigs feeding on waste and excrement were considered impure (Volokhine, 2014). As discussed in the original paper, the presence of *Taenia* eggs in the intestinal lumen of the mummy could support the diagnosis of cysticercosis. The use of fluorescent antibodies, recently allowed the identification of cysticercosis in an Egyptian mummy (Bruschi et al, 2006). Two other publications report *Trichinella* in ancient mummies: in the Cerro El Plomo mummy, an 8–9 years old Inca child of the XVIth century (Rodriguez et al, 2011) and in a member of the Inuit accidentally frozen family of Utqiagvik, Alaska (Zimmerman & Aufderheide, 1984; Cockburn et al., 1998).

In both occurrences, the images were not very convincing even after the use of indirect immunofluorescence for the Inca mummy. Incas were usually vegetarians feeding only occasionally on lama, alpaca or guinea pig meat.

On the opposite, trichinellosis is always frequent in Inuit populations after walrus or bear meat consumption. Diagnosing *Trichinella* in mummies seems very difficult as larvae of this parasite are usually living in muscular cysts surrounded by a collagen capsule and could probably not support the taphonomic processes of mummification. The use of fluorescent antibodies and probably, mainly the use of aDNA amplification could prove the infection.

Further discoveries could be difficult as scientists are now reluctant to perform destructive autopsies. In addition, ethical issues can be discussed as highlighted by Kaufman & Ruhli (2010) in their stimulating paper “Without informed consent? Ethic and ancient mummy research”.

Human Trichinellosis and Treatment

Clinical forms of manifestation of human trichinellosis in Braşov County, Romania, for a period of 30 years

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Our professional interest in human trichinellosis lies for a period of 30 years (1983–2013). We aimed to analyze the clinical forms of manifestation of trichinellosis in 1278 cases recorded in Braşov County, correlated with the average incubation, housing location, gender, age, etc. In Romania, the diagnosis of human trichinellosis is based on clinical diagnostic elements (fever, edema, myalgia), epidemiological, epizootological investigations, and eosinophilia values in dynamic. Specific anti–*Trichinella* IgM and IgG test are currently limited in practice, because they are chargeable or not available.

In the studied cases the average incubation period was 17.25 days, the data were obtained from epidemiological investigations carried out for each case of trichinellosis. Correlating the incubation with the severity of the disease in the mild form (451 cases), the average incubation time was 17 days; the early signs are hardly appreciated by patients as signs of the disease. In moderate forms (713 cases) the average incubation time was 15 days, but unlike the mild one the clinical signs are more obvious alerting the patient to visit the doctor. In moderate–severe forms of the disease (32 cases), the average incubation time was 21 days. Although clinical signs occurred in the context of disease, the clinical diagnosis was not directed toward the parasitosis but towards other diseases. In the severe forms (47 cases) the average incubation period was 16 days; this may be due to the patient's preexisting diseases overlapping trichinellosis.

Mild and severe forms of the disease prevail in urban areas, and the asymptomatic, moderate and moderate–severe in rural areas. In adults moderate, moderate–severe and severe forms of the disease were recorded more frequently, while in children asymptomatic and mild forms occured, because they consume much smaller quantities from the infected meat. In women moderate and severe forms of disease were detected more frequently, and in men the asymptomatic and mild forms were observed more often and probably caused by the damaging effect of alcohol on the *Trichinella* larvae in the intestinal phase of the infection.

High numbers of eosinophil cells were found consistently in all forms of the disease (89.35 %) which confirms the value of this investigation in the diagnosis of trichinellosis, especially in asymptomatic and mild forms of the disease.

Human Trichinellosis and Treatment

Seropositivity to *Trichinella* spp. in Roma population from segregated settlements and in non–Roma population of Eastern Slovakia

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Trichinella spp.is the causative agent of human trichinellosis and circulates predominantly within the sylvatic cycle in Slovakia, with red foxes as main reservoirs of infection. For humans, consumption of pork and wild boar meat present the highest infection risk and has been confirmed as the cause of several outbreaks of disease recorded in last decades (Dubinský et al., 2001, Reiterová et al., 2007; Paraličová et al., 2008). Roma people belong to one of the largest minorities in Europe and in Slovakia estimated number of Roma exceeds 400,000 with approximately one sixth of them living in segregated settlements. Socioeconomic conditions and health of the Roma minority are worse than that of the majority population. Moreover, their eating habits differ from that of the majority population; and consumption of dog meat, meat from cadavers and meat from unknown sources has been recorded occasionally. Therefore the aim of the study was to map the seroprevalence of trichinellosis in the population living in segregated Roma settlements in Eastern Slovakia and to compare it with the majority population.

Antibodies to *Trichinella* spp. were detected in 3 (0.7 %) out of 429 Roma people examined, while nobody (0.0 %) out of 394 non–Roma population was positive. Although, the difference was not of statistical significance (p=0.22), the relative risk of infection in Roma minority was more than 6 times higher (RR=6.43, 95 % CI 0.33–124.10) than in majority citizens. Of three positive, one was 20 years old man and two were women in their 40–ties. Both women suffered from headache and fatigue and in one of them also muscle pain and influenza–like symptoms were present. Positive man did not report the presence of symptoms that could be related to *Trichinella* infection.

Presented results confirmed low seropositivity to *Trichinella* spp. among Roma people and indicate that undercooked meat consumption is not typical in this ethnic minority. However, confirmed higher risk of the infection in segregated settlements with Roma population should be taken into account due to the poor hygiene conditions.

Human Trichinellosis and Treatment

Trichinellosis in Serbia, evidence on long lasting antibody presence – pilot study

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This study presents data regarding 12 subjects involved in two *trichinellosis* outbreaks that took place in Serbia at the end of previous and beginning of this century. Three patients were involved in the outbreak reported in Belgrade, 1997 and 9 were involved in 2002 outbreak in the town of Zrenjanin. For both outbreaks the source of infection was smoked sausage, prepared from uninspected pork harboring *Trichinella* spp. larvae.

All patients involved in this study had clinical signs at the time of the infection and met the case definition criteria for trichinellosis. Patients from Zrenjanin were hospitalized and treated with mebendazole and corticosteroids while the others were ambulatory treated with mebendazole. Current data obtained by questionnaire indicated that 7 patients complained to have myalgia periodically, but the rest of the patients denied existence of any clinical signs regarding trichinellosis.

Three patients indicated that they have noticed some improvement in their health condition during the years after *Trichinella* infection. Two of them emphasized that for many years they did not have even flu, while one patient declared no relapses of herpes simplex infection. Serological investigations performed by indirect immunofluorescent test (IIF) and ELISA assays revealed the presence of specific IgG antibodies against *Trichinella* in the circulation of 10 out of 12 patients (83 %), in particular in sera of all 3 cases from Belgrade and 7 from Zrenjanin.

The IIF titers of positive sera ranged from 1:80 to 1:320. An ELISA test with ES antigen confirmed the presence of specific IgG and absence of specific IgE in same sera samples. The obtained data supports the opinion that infection with *Trichinella* could induce long lasting humoral immune response in humans. Statements from some patients regarding the improvement in their health status in the period post infection, though subjective, draw attention to this phenomenon.

Human Trichinellosis and Treatment

MMP–9 and 2 in human trichinellosis

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Matrix Metalloproteinases (MMPs) are involved in many physiological and pathological processes such as cancer, tissue regeneration/repair and inflammation. Regarding parasitic infections, the role of these proteins has been particularly studied in malaria, neurocysticercosis and angiostrongyloidosis.

Recently, we analysed serum levels of MMP–9 and –2 (gelatinases) in mice experimentally infected with *Trichinella spiralis* or *Trichinella pseudospiralis*, which cause different grades of myositis and we found they significantly increased in the former and, to a lesser extent, in the latter, thus suggesting the possibility that these gelatinases may represent a marker of inflammation. Our aim was to evaluate the levels of MMP–9 and 2 in trichinellosis patients, to assess their possible clinical significance.

Serum samples from *Trichinella britovi*–infected individuals (n=31) and living in the province of Lucca, Tuscany, central Italy, were analysed for MMP–9 and MMP–2 activities. The patients were 11 women and 20 men, infected during November–December 2012 with *T. britovi* following the consumption of raw or undercooked meat of wild boar. The median age was 49 ± 0.33 years (range from 7 to 91). Sera were collected before, and after anti–inflammatory therapy, aliquoted and stored at –20°C until use. Sera from healthy subjects were taken as control group. The gelatinolytic activity of MMPs was analysed by gelatin zymography on 8 % polyacrylamide–SDS gels containing 0.1 % porcine gelatin, under non–reducing conditions. Clear bands corresponding to the digested areas were evaluated with an appropriate software. MMP–9 levels were additionally determined in 15 patients using a commercial ELISA kit for human MMP–9. Differences in the gelatinase activity between the two groups were assessed using a two–tailed Student's *t*–test assuming equal variances. The significance level was set at $P < 0.01$.

The zymographic analysis of the gels showed the presence in serum samples of gelatinase bands at approximately 92–kDa and 72–kDA, corresponding to the pro–enzyme form of MMP–9 and MMP–2, respectively. Areas of lysis of *T. britovi*–infected patients were compared to controls. A significant ($P < 0.01$) increase in gelatinolytic activity in patients compared to the control group was observed for pro–MMP–9 in 25 out of 31. The mean increase in activity was 39.25 % ± 16.67 %. No significant differences were observed for pro–MMP–2 activity. The MMP–9 level detected by ELISA test showed significant concordance with zymographic data (r^2=0.62, $P < 0.003$).

In conclusion, MMP–9 was a reliable marker of inflammation in *T. britovi* patients. On the contrary, MMP–2 did not show significant difference in patients compared to the controls.

4.7 Legislation and Control

Validation of the PrioCHECK® *Trichinella* AAD KIT for the detection of *Trichinella* infections in pigs

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The PrioCHECK® *Trichinella* AAD KIT is a new artificial digestion method, which uses a recombinant serin–endopetidase of the enzyme group subtilisine, instead of pepsin and without the need of hydrochloric acid, to digest muscle tissues for the detection of *Trichinella* larvae. The kit has been validated according to the EURL for Parasites Guidelines approved by the EU member states, which provides the involvement of 5 laboratories. The validation protocol included specificity, sensitivity, robustness and reproducibility assessments. Each of the 5 European laboratories tested 20 meat ball samples of 100 g each, made with minced pork spiked with three encapsulated *Trichinella spiralis* larvae.

Meat samples were digested at 60 °C ± 2 °C for 20 ± 2 min, according to the manufacturer's instructions. The average undigested material on the sieve was 0.9 g (range 0.0 – 4.5). When non–standardized separatory funnels were used and 40 ml of the sedimented digestion fluid were run off, at least one larva was detected in 85 % of the samples. Since false negative results were obtained in two laboratories, a new panel of samples was tested using an unique separatory funnel (Lenz® Squibb NS 29/32, capacity 2,000 ml) in the laboratories. Furthermore, the volume of the run off digestion fluid was increased up to 80 ml. In these conditions, at least one larva was detected in 100 % of the samples.

All detected larvae were dead, but maintained their morphology intact. Single larvae recovered after digestion by the KIT were correctly identified at the species level by molecular identification according to a validated method (multiplex PCR). The KIT is easy to use, the amount of undigested tissues on the sieve is very low, and allows to perform the digestion of muscle tissues even in case of pepsin shortage on the market.

From the safety point of view, the absence of dangerous substances like pepsin and hydrochloric acid decreases the risk of operators to get injured by skin contact or inhalation. Also, because all larvae recovered by the test are dead, the decontamination step is not required when positive samples are detected. However, since larvae obtained after digestion are not alive, the larval movements cannot be of help to recognize the parasite among indigested debris in the sediment. As the amount of undigested debris in the sediment is higher than that obtained by the pepsin–HCl digestion, more accuracy is required during the test execution.

Legislation and Control

Proficiency testing to detect *Trichinella* larvae in meat: Report of nine years of activity at the European Union Reference Laboratory for Parasites

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According to the Commission Regulation 2075/2005, the gold standard for detecting *Trichinella* spp. larvae in meat is the magnetic stirrer method. As this method does not allow the use of internal controls to ensure the proper execution of the test, an appropriate training of laboratory staff and a regular assessment of the testing procedures are required. Since 2007, the European Union Reference Laboratory for Parasites (EURLP) organizes an annual proficiency testing (PT) for National Reference Laboratories (NRL) for Parasites of EU countries, to evaluate their performance in conducting the test.

Samples consist of 35 or 100 g of minced pig or horse meat spiked with *Trichinella spiralis* muscle larvae. The PT was passed when the participant correctly identified all positive samples (qualitative evaluation). A quantitative evaluation based on the number of recovered larvae, was also included.

For each sample, the difference between the expected and reported number of larvae was indicated and the absolute mean of this difference was calculated over the total number of samples. To compare the results over time, the relative mean between expected and observed count was calculated. The overtime comparison of qualitative results for the 2007–2015 period showed that 22 (62.9 %) labs passed the PT in the first round and continued to obtain positive results, 5 (14.3 %) labs passed the PT session after one or more failures in the first rounds, and 8 (22.8 %) labs alternated positive and negative results. The percentage of labs reporting false negatives decreased from 37.5 % in 2007, to 11.4 % in 2015, and the percentage of labs, which overestimated the number of larvae decreased from 25 % in 2007 to 6.2 % in 2015. False positive results were obtained by two laboratories in 2010, by one laboratory in 2012 and in 2013.

The comparison of the overall relative mean difference values showed a constant improvement in the PT results from 2007 to 2015. The decreased value of the overall mean difference observed implies an increased accuracy in the analysis. Yet, it can also be explained by the fact that the number of samples in the PT panel and the number of larvae per sample were reduced overtime potentially leading to a less mistakes during larvae counting. Despite the good results achieved during the 2007–2015 period, there are still participant laboratories reporting false negatives and, consequently, there is still the need for continuous monitoring.

Legislation and Control

Sedimentation funnel as a new source of error in official *Trichinella* examination

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Official controls for *Trichinella* in meat are the basic legal principle in meat hygiene and a milestone of preventive consumer protection within the European Union. According to Regulation (EC) No 2075/2005 the magnetic stirrer method for pooled sample digestion (MSM) is stipulated as the reference method. In particular, therefore, this method must comply with the highest standards of analytic quality. Although a precise description on how to conduct the MSM correctly is laid down in the aforementioned regulation, only limited information about the required labware quality is stipulated.

A publication in 2007 found that the application of plastic labware during sedimentation can cause larval losses (Vallee et al., 2007). This may be attributed to interactions between the material surface and the *Trichinella* larvae (L1) most likely based on ad– and absorption or mutual interactions. However, this error source was not systematically studied. So far glass separation funnels were never considered to cause larval losses.

We studied larval recovery using different glass sedimentation funnels and describe for the first time serious larval losses. Spiked *Trichinella*–negative digestion fluid was examined for L1 after 30 minutes sedimentation using five different sedimentation funnels. One funnel was newly purchased and unused. A second funnel was used only a few times before and initially introduced shortly before our participation in ring testing. Two other funnels had already been applied in daily routine examination for several years. An unused fifth funnel (F5), identical to the newly purchased one was obtained after the main experiment was conducted and tested separately. The experiments were performed 25 times per funnel (F5, 12 times), spiked with 20 L1 each. With a recovery rate of more than 90 % three separation funnel showed a comparatively good performance (94.4 %, 91.1 %, and 95.6 %, respectively). Moderate test results were achieved with one funnel (69.4 % recovery rate). The fifth funnel showed a constantly bad performance. From 240 L1 spiked only 84 L1 were detected (35 %).

Worst results were obtained with 3 out of 20 L1 (15 %). An extension of the sedimentation time to 60 minutes and the release of a second 40 ml volume led to a further recovery of 51 % of the missing larvae.

Our study shows for the first time that separation funnels made of glass can cause serious larval loss. Therefore, individual testing of the separation funnels should be recommended as part of each's laboratories' quality assurance system.

Legislation and Control

A current status of evidence on *Alaria* spp. mesocercariae in game

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Distomum musculorum suis (DMS), the mesocercarial stage of the trematode *Alaria alata*, can cause severe damage within their hosts, and since several reports about cases of human larval alariosis have been published, it became apparent that infected game animals and in particular wild boars are a potential source of infection for both, humans and animals. Odening (1963) reported a massive *A. alata mesocercariae* infection during an in vivo experiment with rhesus monkeys (*Macaca mulatta*). He identified DMS in several vital organs including the heart. These findings represented clear evidence that a host closely related to humans can become infected with the parasite.

For the first time, a generalized infection of a person was described by Freeman et al. (1976) and Fernandez et al. (1976). A 24–year–old Canadian male complained of tightness in the chest and abdominal symptoms. After initial symptoms, the patient died in hospital. The Federal Office for the Environment (FOEN) in Switzerland categorized *A. alata* as a stage 2 risk (Z) for parasites with zoonotic potential. In its statement, the German Federal Institute for Risk Assessment (BfR) pointed out that a human risk could not be precluded and that a suitable method for the detection of this parasite was missing (BfR 2007). Thus, we developed a totally new analytical procedure, the so called *A. alata mesocercariae* migration technique (AMT), for the reliable detection of this parasite in game meat. The AMT provided a comparable or even higher sensitivity as the magnetic stirrer method for the detection of *Trichinella* during interlaboratory tests and was already successfully applied in several studies under field conditions (Riehn et al. 2010, 2013).

Here we present results that further demonstrate the official method for the detection of *Trichinella* spp. in muscle tissue (Annex I, Chapter I, Regulation (EC) No. 2075/2005 (TIM)) to be highly unsuitable for the detection of *A. alata mesocercariae* in meat. Using TIM, we have to expect a so far unknown but substantial number of false–negative results. AMT is a simple, robust, highly applicable, low–cost, and fast method. The German Federal Ministry of Food and Agriculture has recognized this analytical procedure following the conclusion of the research project 2012 as an official procedure. In conclusion we recommend the application of AMT in all cases where the presence of *A. alata mesocercariae* in game meat cannot be precluded on the basis of epidemiological evidence.

Legislation and Control

Cooking methods and infection with *Trichinella papuae* in Papua New Guinea

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Wild pigs (*Sus scrofa*, considered to be hybrids between *Sus scrofa vittatus* and *Sus celebensis*) are infected with *Trichinella papuae* in two districts of Papua New Guinea, Morehead, Western Province, and Kikori, Gulf Province, but only the inhabitants of Morehead show anti–*Trichinella* IgG in their sera.

People of both districts have similar customs and livelihoods; they favour wild pig meat as a source of protein and occasionally eat tidbits of raw meat, but there are differences in the preferred methods of cooking. Boiling is the predominant method of cooking meat in Kikori, followed sequentially by roasting/grilling, using bamboo tubes, nipa palm leaves, and earth–oven or 'mumu'. In Morehead, 'mumu' is the most common method of cooking, followed by roasting/grilling and boiling. Efficient 'mumu' cooking requires the 'mumu' to be made in a pit, using pre–heated river stones.

As there are no river stones in Morehead, people use substitute materials, like, sun–dried clay balls, pieces of termite mounds and small rough stones that are less efficient than river stones in retaining heat. Also, a Morehead 'mumu' is made on the surface of the ground. Those villagers in Kikori that use 'mumu' have access to river stones and use a pit. It is suggested that the 'mumu', as used in Morehead, places people at risk of infection with *T. papuae*, while the cooking methods, including 'mumu', as practiced by Kikori people prevent infection.

Legislation and Control

Validation of digestion assay based on results of proficiency comparison results 2007–2014 in Poland

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Council Regulations (EC) No 853/2004, No 854/2004 and No 882/2004 of the European Parliament laid down hygienic rules for production of food of animal origin together with requirements regarding official controls. This lead to the specific requirements for *Trichinell*a controls set up in Commission Regulation (EC) No 2075/2005 of 5 December 2005. For the purpose of the official control of *Trichinella* the magnetic stirrer method for pooled–sample digestion was recommended as a reliable method for routine use and as reference method.

According to mentioned regulation, all personnel involved in the examination of samples to detect *Trichinella* should participate in a quality control programs and a regular assessment of the testing, recording and analysis procedures used in the laboratory. Proficiency testing (PT) is an accepted tool for quality assurance. It is also an essential component for accreditation bodies (ISO 17025), which require PT complement to prove competence of laboratory. Both basic quality assurance and PT programme are widely used to improve laboratory performance and ensure food safety.

The current study presents the results of validation of digestive assay based on results of PT organized by Polish NRL. Presented data were collected in 2007–2014 as results of PT in Poland. Collected data enable us to establish parameters characterizing the method and so called field validation. For every round of PT, different parameters characterized the method: the accuracy (AC), specificity (SP) and sensitivity (SE) were evaluated according to the qualitative part of the EN ISO 16140. Within last seven years veterinary laboratories have examined over 18 000 PT samples, with mean accuracy 96 %, sensitivity 95 % and specificity 97 % with confidence interval p=95 (mean upper limit 98,15 and mean lower limit 95,1 %). The detection limit was established on the base of % of positive results versus all samples on the basis of 1 larva per sample. For the detection limit with 1 larvae per sample, 60 % of laboratories (375 out of 533) obtained positive results.

We can assume that organized PTs were helpful tool to increase quality of laboratory work and good source of information for method validation.

Legislation and Control

The study of optimized conditions of artificial digestion method for inspection of *Trichinella spp.*

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The International Commission on Trichinellosis (ICT) makes uniform standards and rules for digesting and collecting *Trichinella* spp. However, the biological characteristics of 12 species of the genus *Trichinella* spp. are different, therefore the methods of digestion and collection exist a certain differences.

Interestingly, we found that the *Trichinella murrelli* muscle larvae (ML) stretched, moved and floated in process of the collection, so it slowly deposited and many *T. murrelli* larvae may be recovered by the extension of sedimentation time. In this study, the BALB/c mice were infected with muscle larvae of 12 species of the *Trichinella* spp., respectively, and ML recovered at 40 days post–infestation (dpi) using the ICT–digestion method. Meanwhile, the larvae recovery rate was calculated and compared under the condition of 4 °C and 25 °C at different time points (20 min, 30 min, 45 min, 60 min and 75 min), and the optimized conditions for the larval recovery from digestion were evaluated.

The results showed that the larva recovery rate of different *Trichinella* spp. species and genotypes was different and depended on digestion time and temperature, and the optimized conditions (4 °C). A higher larval recovery rate was achieved if sedimentation time was at least 45min.

This study demonstrated that the current ICT digestion method may have shortcomings, which may lead to false negative result for *Trichinella* detection, and ICT should pay attention for this.

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Legislation and Control

Trichinellosis in Baden–Württemberg

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Domestic pigs, wild boars and wild carnivores are considered the main vectors of *Trichinella* by acquiring infection through scavenging and cannibalism. In Germany trichinellosis, ranking among the worldwide most important parasitic zoonoses, has become very rare.

The examination of *Trichinella* as part of the official meat inspection is required by law in the Member States of the EU and described in detail in Regulation (EC) No 2075/2005. Therefore, meat of pigs, wild boars and other animals susceptible for *Trichinella* are fit for human consumption if the examination of *Trichinella* yields a negative result.

If housing conditions for domestic pigs are officially recognised as controlled, carcases and meat of domestic pigs may be exempt from *Trichinella* examination in the EU since the detection rate tends toward zero for many years. However, care should be taken regarding undercooked meat or sausages produced from pigs originating from smaller outdoor farms and slaughtered for own consumption.

Apparently there is no systematic examination for home slaughtered pigs and hunted wild boars in many countries. At the beginning of 2015 eight people in Baden–Württemberg have suffered from trichinellosis after eating an air–dried paprika salami from Serbia made for own consumption and imported to Germany during private travel. Examinations of samples from the paprika salami at the BfR and STUA Aulendorf – Diagnostikzentrum confirmed a massive *Trichinella* infestation. Clinical symptoms of the infected persons ranged among others from diarrhea partially followed by obstipation, edema of the face, increasing pains in the muscles, attacks of fever to a general feeling of illness.

In Baden–Württemberg the finding of *Trichinella* in a sample of wild boar´s meat in 2012 underlines the significance of the *Trichinella* examination as important preventive measure in term of consumer health protection. The quality of *Trichinella* examination is regularly checked by close cooperation (e.g. audits) of the Veterinary offices, the STUA Aulendorf – Diagnostikzentrum and the CVUA Freiburg. Twice a year, the STUA – Diagnostikzentrum performs proficiency tests for the detection of *Trichinella* in meat samples with about 200 participants.

Legislation and Control

Monitoring of *Trichinella* in pigs – sample size estimation

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In Germany findings of *Trichinella* spp. in meat inspection are extremely rare especially in domestic pigs. Surveillance data of recent years show a very low prevalence (2003–2012: 489 mill. pigs were tested: 8 cases, prevalence<10^{-7}). The positive tested pigs were kept in small private holdings. Thus, the exposure of consumers through infected meat is considered negligible. Between 2001–2014, 108 trichinellosis cases have been reported in humans.

Currently, systematic *Trichinella* testing of all slaughtered pigs is mandatory in Germany. However, there are considerations to replace systematic testing with a risk–based monitoring. In this regard digestion method, ELISA, Western blot and also a sequential combination of ELISA and Western blot were compared with respect to the required sample size for a *Trichinella* monitoring.

Both different herd sizes (10, 100, 1000, 10000 pigs) as well as different test sensitivities on herd level were taken into account for sample size calculation at various prevalence assumptions. The herd sensitivity is the probability to classify a herd in which at least one infected animal occurs as affected.

The results show that the monitoring of rare events such as *Trichinella* infections requires large sample sizes and corresponds to high expenditures. The validity of the currently available classical and serological diagnostic test procedures for correct herd classification within the scope of a monitoring system for fattening pigs from herds with a negligible risk of *Trichinella* infection is limited.

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